

AWARD NUMBER: W81XWH-13-2-0072

TITLE: Brain Immune Interactions as the Basis of Gulf War Illness: Gulf War Illness Consortium (GWIC)

PRINCIPAL INVESTIGATOR: Kimberly Sullivan, Ph.D

CONTRACTING ORGANIZATION: Boston University Medical Campus
Boston, MA 02118

REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE			<i>Form Approved</i> <i>OMB No. 074-0188</i>	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. Agency Use Only (Leave blank)	2. Report Date October 2016	3. Report Type and Period Covered Annual report 30 Sep 15 - 29 Sep 16		
4. Title and Subtitle Brain Immune Interactions as the Basis of Gulf War Illness: Gulf War Illness Consortium (GWIC)			5. Award Number W81XWH-13-2-0072	
6. Author(s) Kimberly Sullivan, Ph.D. email: ttt@bu.edu			8. Performing Organization Report Number (Leave Blank)	
7. Performing Organization Name Boston University Medical Campus Boston, MA 02118 E-Mail: ttt@bu.edu				
9. Sponsoring/Monitoring Agency Name and Address U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. Sponsoring/Monitoring Agency Report Number (Leave Blank)	
11. Supplementary Notes (i.e., report contains color photos, report contains appendix in non-print form, etc.)				
12a. Distribution/Availability Statement (check one) Approved for public release; distribution unlimited			12b. Distribution Code (Leave Blank)	
13. Abstract (Maximum 200 Words) The primary function of the Gulf War Illness (GWIC) consortium is to identify the pathobiological mechanisms of Gulf War Illness. The ultimate goal is to discover and characterize biomarkers of Gulf War illness and then identify targeted treatment strategies. The GWIC allows for the development of multidisciplinary collaborations targeting suspected brain-immune signaling alterations in GWI. The GWIC consortium central hypothesis identifies chronic neuroinflammation as an end result of initial glial activation and subsequent priming of glial responses that cause a chronic activation loop of stronger and longer proinflammatory signaling effects between the immune system and the brain. The GWIC includes both clinical (human) and preclinical (animal and cell) studies and researchers in the 10 funded sub-studies. These studies are incorporating sufficient overlap of scientific content area to inform each other in a bench-to-bedside-to-bench approach. Results to date from the preclinical (animal) studies suggest a strong neuroinflammatory component to the illness model and provide leads for treatment development approaches in the animal model before translation to the clinic. Clinical study recruitment has begun and has shown preliminary correlations between proinflammatory cytokine markers and behavioral and neuroimaging outcomes. Larger samples sizes will continue to make these inter-relationships more clear.				
14. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award) Gulf War Illness, consortium, CNS, innate immunity, cytokines, MRI neuroimaging, cognitive deficits, pesticides, DFP, sarin			15. Number of Pages (count all pages including appendices) 47	
17. Security Classification of Report Unclassified			16. Price Code (Leave Blank)	
18. Security Classification of this Page Unclassified		19. Security Classification of Abstract Unclassified		20. Limitation of Abstract Unlimited

NSN 7540-01-280-5500

Standard Form 298
(Rev. 2-89)

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Overall Project Summary.....	4
4. Key Research Accomplishments.....	37
5. Conclusion.....	38
6. Publications, Abstracts, and Presentations.....	39
7. Inventions, Patents and Licenses.....	40
8. Reportable Outcomes.....	42
9. Other Achievements.....	42
10. References.....	45
11. Appendices.....	47

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The primary function of the Gulf War Illness (GWIC) consortium is to identify the pathobiological mechanisms of Gulf War Illness. The ultimate goal is to discover and characterize biomarkers of Gulf War illness and then identify targeted treatment strategies. The GWIC allows for the development of multidisciplinary collaborations targeting suspected brain-immune signaling alterations in GWI. The GWIC consortium central hypothesis identifies chronic neuroinflammation as an end result of initial glial activation and subsequent priming of glial responses that cause a chronic activation loop of stronger and longer proinflammatory signaling effects between the immune system and the brain. The GWIC includes both clinical (human) and preclinical (animal and cell) studies and researchers in the 10 funded sub-studies.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Gulf War Illness, consortium, CNS, innate immunity, cytokines, MRI neuroimaging, cognitive deficits, pesticides, DFP, sarin, CORT, genetics, objective biomarkers, treatment development

3. **OVERALL PROJECT SUMMARY:**

INTRODUCTION

Background. Twenty five years after the 1991 Gulf War, 25-30% of the nearly 700,000 U.S. troops who served in the war still suffer from the debilitating symptomatic illness known as Gulf War Illness (GWI) (White et al., 2016; RAC, 2008, 2014, IOM, 2010). A growing body of evidence indicates that GWI is associated with diverse central nervous system (CNS) and immune alterations, but the specific pathobiological processes driving GWI symptoms have not been clearly elucidated (Zhang et al., 1999; Sullivan et al., 2003; Heaton et al., 2007; Toomey et al., 2009; Whistler et al., 2009; Broderick et al., 2011; Chao et al., 2011; Sullivan et al., 2013; White et al., 2016; Abou-Donia et al., in revision). Animal studies indicate that a chronic CNS inflammatory state can develop in response to an insult—chemical injury, infection, or physical trauma (including mild traumatic brain injury)—that mobilizes CNS defense systems via activation of glia, the brain’s primary immune response cells, and release of chemical messengers that precipitate a complex of “sickness behavior symptoms” identified by measures of impaired memory and learning, increased pain sensitivity, and persistent fatigue, a symptom complex similar to that of GWI (Rathbone et al., 2015; Banks & Lein, 2012; Watkins et al., 2007; 2009; Zhang et al., 2010). Recent studies have also demonstrated CNS inflammatory effects of GW-related exposures and additional immune and cellular processes that plausibly explain the mechanisms contributing to the full spectrum of GWI symptoms (O’Callaghan et al., 2015; Milligan et al., 2009; Rivest et al., 2009; Spradling et al., 2011).

Consortium Management and Expertise. This multidisciplinary collaboration brings together established GWI researchers, and leading experts in brain-immune processes associated with neurotoxicology and neuroinflammation, damage to white matter and axonal transport, immunology, and immunogenetics. This team has designed a body of interrelated studies linked together by a cohesive model of ‘brain-immune interactions’ as the basis for GWI. The consortium is led by Dr. Kimberly Sullivan, at Boston University (BU), whose extensive background in GWI research includes contributions in identifying effects of Gulf War exposures on brain structure and function (Sullivan et al., 2003; Sullivan et al., 2013; Yee et al., 2015). BU serves as the Coordinating Center for the Gulf War Illness Consortium (GWIC) and provides the Administrative and Data Management Cores (figure 1). The consortium also includes a Preclinical Core, consisting of experts at five sites who are working collaboratively to characterize the persistent neurological and immune effects of GW exposures at the physiological, tissue, and cellular levels. This is done in parallel

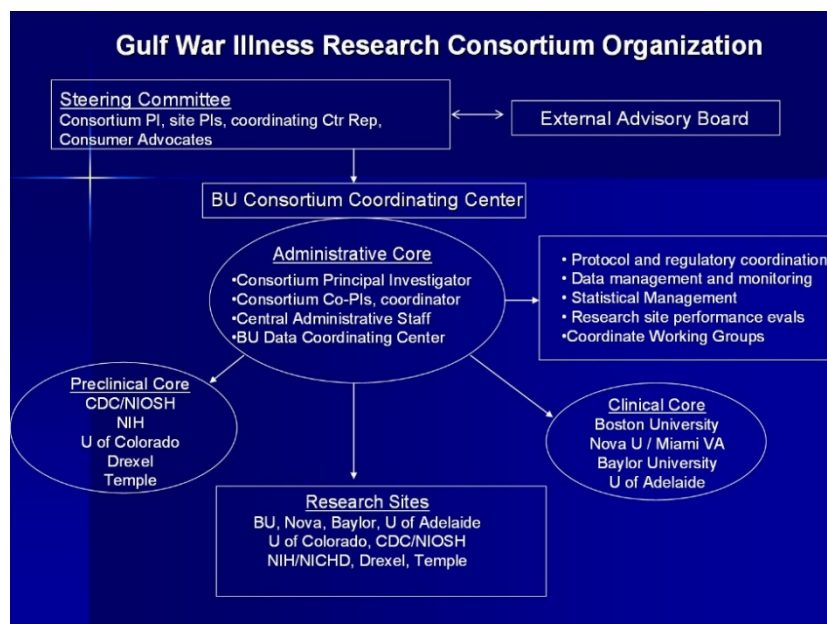


Figure 1. GWI Consortium schematic Organizational Chart

with human studies conducted by the Clinical Core at three recruitment sites (and two additional laboratory sites) to characterize the specific profile of brain, immune, and genetic measures that distinguish veterans with GWI from healthy controls. The GWIC Steering Committee and External Advisory Committee monitors research progress and findings, and advises on research modifications and follow-up.

Objective. The primary objective of the Boston GWI consortium is to provide a cohesive understanding of the pathobiological mechanisms responsible for the symptoms of GWI in order to provide a rational and efficient basis for identifying beneficial treatments and diagnostic markers.

Research Plan. The consortium is undertaking a coordinated series of clinical and preclinical studies aimed at providing a comprehensive understanding of the pathobiology of GWI. This includes clinical case-control studies conducted in parallel at 3 subject recruitment sites—Boston, Miami, and Central Texas—that include a total of 300 Gulf War veterans. Clinical assessments include a) advanced neuroimaging protocols (MRI, DTI, fMRI, PET) that assess brain volumetrics, white matter integrity, and CNS inflammatory indicators, b) neuropsychological assessment of cognitive function, c) blood levels of cytokines and other immune signaling molecules, d) genetic expression of immune markers, e) pilot assessment of cerebrospinal fluid levels (CSF) of cytokines and neurotransmitters (in subgroup of Boston cohort), f) immunogenetic markers of innate immune responsivity, f) longitudinal assessment of brain-immune measures (Texas cohort only). Parallel preclinical studies are evaluating persistent effects of GW neurotoxins *in vitro* and in rodent models of GWI. Preclinical studies are evaluating cellular effects of GW neurotoxins on a) axonal transport, b) glial cytokine production, c) neurotransmitter signaling, d) myelination, and e) oligodendrocyte proliferation. Animal studies are determining the effects of GW exposures on: a) priming and maintaining glial activation, differentiating effects on astrocytes vs. microglia, b) glial activation in relation to development of learning impairment and chronic pain sensitivity, c) brain and blood levels of proinflammatory cytokines, and d) genetic expression of immune and inflammatory markers in brain and blood. Findings from clinical and preclinical studies will be compared and used to identify specific brain-immune pathways that can be targeted for intervention by a variety of glial modulating and other currently available treatments. Treatment compounds will be tested in animal models to determine their effectiveness for resolving or ameliorating the pathobiological processes associated with GWI. Figure 1 represents the hypothesized mechanisms for GWI that will be tested by this planned series of preclinical and clinical experiments.

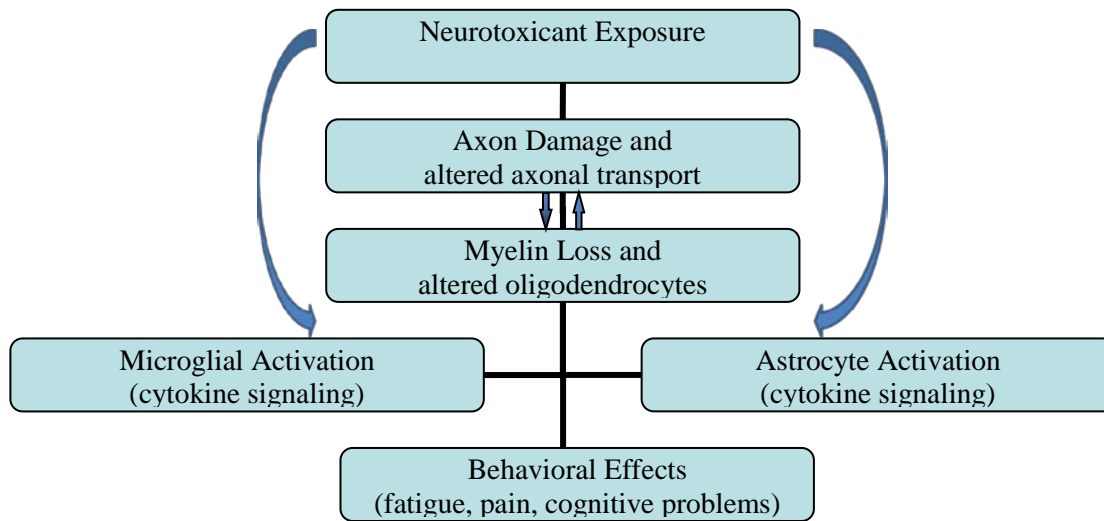
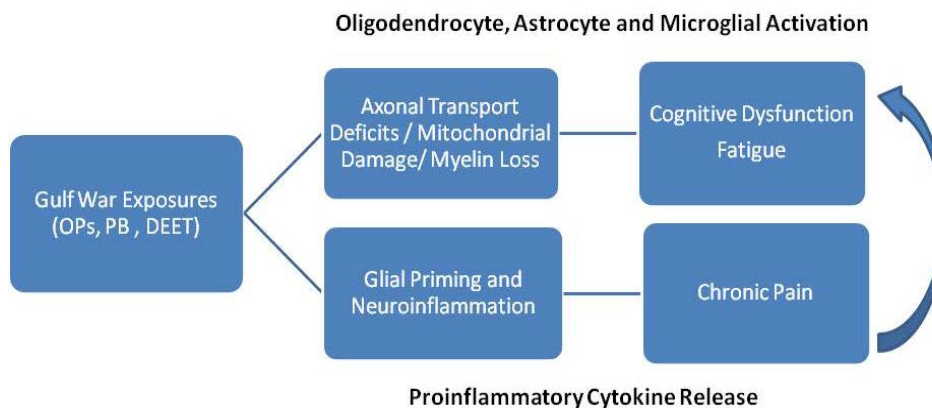


Figure 2. Schematic Representation of Hypothesized GWI Mechanisms

The GWI consortium central hypothesis identifies chronic neuroinflammation as an end result of initial glial *activation* and subsequent *priming* of glial responses that cause a chronic activation loop of stronger and longer proinflammatory effects between the immune system and the brain. Figure 2 below represents the integrated theory of GWI that will be tested in the consortium studies.

INTEGRATED THEORY OF GWI



The overall aims of this integrated multidisciplinary consortium scientific focus are to (1) To identify validated markers of GW illness by using state of the art neuroimaging, behavioral, genetic and blood markers of neuroinflammatory activation in both clinical and preclinical models that will elucidate targeted and validated treatment strategies (2) To create a Neuroinflammation Risk Profile for GWI (3) To identify viable mechanistic treatments based on identified pathophysiological pathways of GWI that have been validated in preclinical treatment models.

BODY

The approved statement of work for the entire study period is below:

STATEMENT OF WORK

Table 1. Brain-Immune Interactions as the Basis of Gulf War Illness: Gulf War Illness Consortium

Task 1. Obtain necessary authorization prior to initiation of human subjects' and animal studies research (months 1-8)
1a. Attend pre-award meeting with CDMRP GWIRP program staff
1b. Obtain final Institutional Review Board (IRB) approval for clinical research sites at Boston University School of Public Health (BUSPH), Baylor University and Miami VA/Nova University for protocols and advertisements
1c. Obtain final DOD Human subjects Research Protections Office (HRPO) approvals
1d. Obtain data use agreement from Hines VA for stored blood sample study
1e. Obtain final protocol approval by the respective Institutional Animal Care and Use Committees (IACUC) approval for the preclinical animal research sites at Center for Disease Control/NIOSH, National Institutes of Health, Drexel University, Temple University and University of Colorado
1f. Complete hiring of necessary staff and ensure all mandatory IRB and IACUC research related trainings are completed by all staff members
Task 2. Preparation for consortium clinical studies (months 1-9)
2a. BUSPH Data Coordinating Center (DCC) will create website, data collection forms, specimen tracking system and databases for the entire consortium including all preclinical and clinical sites.
2b. Develop manuals for the neuropsychological testing protocol, imaging protocols, specimen collection protocols and recruitment.
2c. Train researchers and staff on protocols and quality control measures for the clinical and preclinical studies.
2d. Obtain stored blood samples from Hines VA study and send to Miami VA for analysis.
Task 3. Preparation for consortium preclinical studies (months 9 - 24)
3a. Prepare rat dosing models at CDC and distribute to other sites at NIH, Drexel, Temple and U-Colorado for planned studies of axonal transport, myelin integrity and learning and pain assessments.
3b. Develop co-cultures of rodent oligodendrocytes in cell culture chambers for electrical stimulation of axons and development of myelination in vitro at NIH.
Task 4. Perform preclinical cell and animal studies (months 9-42)
4a. Assess for axonal transport integrity in rodent and cell models exposed to either GW-relevant neurotoxins or cytokines (Drexel - 30 Sprague Dawley rats, Temple - 27 Sprague Dawley rats).
4b. Assess for myelin integrity in rodent and cell models exposed to either GW-relevant neurotoxins or cytokines (NIH – 624 NIH/S mice and 208 rats).
4c. Assess whether persistent priming of neuroinflammation occurs chronically with GW-relevant neurotoxins and intermittent corticosterone exposure to model the chronic nature of GWI (CDC – 100 C57BL/6 mice).

4d. Assess the relative contributions of astrocytes and microglia in rodent GWI neuroinflammatory models in order to identify which glial markers will provide the best candidate “drugable” targets (CDC 40 C57BL/6 mice; 40 ALDH1L1 mice; 40 B6.129-Cx3CR1 mice).
4e. Assess the relationship between behavioral testing of learning and memory and enhanced pain, in rodent GWI neuroinflammatory models by assessing hippocampal functioning with a fear conditioning task (U-Colorado – 120 rats).
4f. Compare central and peripheral markers of neuroinflammation in brain tissue and blood samples from GWI neuroinflammatory rodent models (CDC – 60 rats, Nova).
4g. Compare the effectiveness of several relevant preclinical treatments for GWI in cell and animal studies, including inflammatory glial activation modulators, antioxidants, and neuroprotective peptides (Drexel, Temple, CDC, U-Colorado)(20 animals per treatment).
Task 5. Screening, recruitment and assessment of Gulf War veterans from three sites (months 9-42)
5a. Obtain informed consent from potentially eligible GW veterans
5b. Assess subjects by obtaining demographics, medical history, self-report questionnaires, neuropsychological testing, brain imaging and blood draw and saliva samples.
5c. Upload neuroimaging data to BUSPH for post-processing of MR images and for data analysis.
5d. Score neuropsychological tests and upload summary data to DCC for entry, cleaning and analyses.
5e. Send blood and saliva samples to Nova University for analysis of cytokine and chemokine panels and cortisol measurements.
5f. Send additional saliva samples to University of Adelaide for genetic polymorphism analysis
5g. Conduct preliminary analyses of clinical data
Task 6. Recruitment and assessment for Boston CSF and PET studies (months 24-42)
6a. Perform lumbar punctures to obtain cerebrospinal fluid markers of neuroinflammation in 50 GW veterans.
6b. Perform positron emission tomography (PET) scanning with novel EAAT2 ligand in partnership with RIO pharmaceuticals in 15 GW veterans.
6c. Perform FDG-PET scan imaging with 30 GW veterans after a computerized CPT cognitive challenge task.
Task 7. Interim Analyses, Grant Submission, and Annual Reporting (Months 18-42)
7a. Data entry of all questionnaires, evaluations and quality control measures will be ongoing
7b. Interim Statistical analyses of data obtained from cognitive evaluations, blood markers, neuroimaging and questionnaire data will be performed periodically.
7c. Grant submissions to relevant funding agencies for further collaborative studies based on initial results and preliminary data targeted toward treatment strategies will be ongoing.
7d. Annual reports of progress will be written.
Task 8. Final analysis and Report Writing (months 42-48)
8a. Statistical analyses comparing brain MRI volumetrics, cognitive functioning, health symptom report and cytokine/chemokine markers in veterans with and without GWI
8b. Statistical analyses of correlations between clinical and preclinical

neuroinflammatory markers of GWI models
8c. Perform longitudinal assessments of imaging, cognitive, health symptom and cytokine functioning in veterans with and without GWI
8d. Perform validation analysis studies of identified biomarkers of GWI using an unrelated sample of stored blood and cognitive health symptom data from a prior CSP study.
8e. Write final study report
8f. Present findings at scientific meetings
8g. Prepare manuscripts for submission
8h. Write grant proposals based on consortium findings and identified treatment avenues for GWI.

The statement of work for year 3 is inclusive of Tasks 1-7 above. The statement of work for year 3 primarily describes the completion of the start-up phase of the 10 sub-studies including obtaining local and funder institutional review approvals for animal and clinical studies as well as establishing dosing models for cell and animal studies and finalizing clinical protocols for neuropsychological assessments, blood, saliva and CSF samples and neuroimaging sequences. In addition, in year 3, the plan was to have cell and animal studies underway and reporting initial results. The plan was also to continue with subject recruitment for the clinical studies and to recruit 145 study participants for the study protocol including cognitive evaluations, interviews, neuroimaging and specimen collection. Progress toward completing each task is listed below.

TASK 1. OBTAIN NECESSARY AUTHORIZATION PRIOR TO INITIATION OF HUMAN SUBJECTS' AND ANIMAL STUDIES RESEARCH (MONTHS 1-8)

Task 1a. Attend pre-award meeting with CDMRP GWIRP program staff

Due to delays in funding the consortium as a result of the government shutdown, the pre-award meeting was held in February 2013 and was considered a post-award meeting. The meeting included an overview of study hypotheses and plans as well as a review of the consortium administrative and core center structure. The Consortium PI, Dr. Sullivan and other steering committee members were present at the meeting in addition to CDMRP commanders, grants officer's representative (GOR) and administrative staff. Required External Advisory Board (EAB) meetings have also begun to meet with the first meeting being held in September 2014. Subsequent EAB meetings were held in April 2015, October 2015, and May 2016. The EAB provided helpful suggestions and comments for study progress and discussions for future meetings that will occur semi-annually during the consortium funding period.

Task 1b. Obtain final Institutional Review Board (IRB) approval for clinical research sites at Boston University School of Public Health (BUSPH), Baylor University and Miami VA/Nova University for protocols and advertisements

IRB documents have been submitted and approved for all three clinical sites. Miami VA/NOVA University, and Boston University (BU) have submitted IRB protocols and received local IRB approvals. Baylor Medical College has received local IRB approval and is awaiting HRPO approval in order to begin subject recruitment. University of Adelaide received exempt status from their local IRB.

Task 1c. Obtain final DOD Human subjects Research Protections Office (HRPO) approvals

HRPO submissions have been submitted for all three sites and approved for Miami VA/NOVA University, Boston University and Baylor Medical College study protocols.

Task 1d. Obtain data use agreement from Hines VA for stored blood sample study

All relevant blank study forms from CSP 458 have been obtained and reviewed in order to generate a definition of CMI based on the Kansas definition. GWIC investigators have finalized the definition to compare how many subjects meet criteria for the CDC definition of CMI, the Kansas definition or both. This will inform the selection of the blood samples to analyze. Once selected, blood samples can be sent to the Miami VA (Dr. Klimas) and the final draft of the DUA will be submitted (exactly which variables will be used in the DUA needs to be specified).

The current update is that GWIC has been recently informed by the Hines VA that they no longer can release the de-identified data set to GWIC investigators because of a re-interpretation by VA Central Office which states that such a release would have had to be included in the consent form. However, Hines itself can perform the data analysis in the budgetary funds that were already allotted for this sub-study. The previous DUA needs to be re-written since they are no longer going to release the actual data, but they still require a DUA. Hines will draft a revision of the DUA to reflect the new arrangement and submit to Drs. Toomey, Sullivan, and GWIC investigators to submit for final approval. Once Hines can apply the algorithm for the Kansas definition of GWI, we can begin sending blood samples to Dr. Klimas at the Miami VA. Dr. Klimas will perform cytokine analysis and send results to Hines. Hines will now conduct the statistical analyses.

Task 1e. Obtain final protocol approval by the respective Institutional Animal Care and Use Committees (IACUC) approval for the preclinical animal research sites at Center for Disease Control/NIOSH, National Institutes of Health, Drexel University, Temple University and University of Colorado

All local IACUC approvals have been obtained from CDC, NIH, Temple and University of Colorado. BU offsite IACUC approvals have been obtained for all animal study sites. ACURO final approvals have also been obtained for all pre-clinical sites and renewals are submitted for approval as they are required for 3-year re-writes. Some issues have arisen for the CDC and U-Colorado sites regarding more animal deaths than initially anticipated resulting in expiration of IACUCs. This required planning and consultation with CDC and U-Colorado veterinarians for renewed approvals of IACUCs. The new animal care plans have now been approved by local IACUCs and have been submitted to ACURO for review and approval.

Task 1f. Complete hiring of necessary staff and ensure all mandatory IRB and IACUC research related trainings are completed by all staff members

Hiring of local post-docs and research assistants has been ongoing for each site. BUSPH has hired the consortium research assistant and Dr. O'Callaghan recently hired several post-doctoral associates. All current staff have completed IRB and IACUC trainings necessary for their work with animal and human studies. The Miami site has hired and trained an additional research assistant.

TASK 2. PREPARATION FOR CONSORTIUM CLINICAL STUDIES

The consortium coordinating center and Administrative Core at Boston University has led many monthly web and in-person meetings to prepare for the clinical studies once all institutional approvals were obtained. A significant amount of time and effort was devoted to obtaining all required study and test administration materials and to developing centralized web-based data collection materials for the consortium studies. Table 2 lists these planning meetings. Smaller working group meetings were also held during the past year to plan for particular consortium topic areas. The Working Groups are described in Table 3. Since subject recruitment has begun, considerable time has been spent with training and quality control assurance meetings of clinical staff to ensure consistent inter-rater reliability and to reduce any administration drift from standard testing and scoring procedures. This will continue as the third study site in Texas begins study recruitment efforts.

Table 2. GWIC Monthly Planning and EAB Meetings

Date	Type of Meeting	Discussion Items
10-01-2015	In-person Boston	➤ Data coordination update
10-07-2015	Monthly web meeting	➤ Pre-clinical and clinical updates, prep for EAB mtg
10-28-2015	External Advisory Board meeting in Ft. Detrick, MD	➤ Pre-clinical and clinical updates and preliminary data
11-04-2015	Monthly web meeting	➤ Pre-clinical and clinical study updates
01-12-2016	Clinical planning meeting	➤ Meeting with radiology team for PET study
01-13-2016	Monthly Web-Meeting	➤ Pre-clinical and clinical study updates
02-03-2016	Monthly Web-meeting	➤ Pre-clinical and clinical study updates
03-02-2016	Monthly Web-meeting	➤ Pre-clinical and clinical study updates
04-06-2016	Monthly Web-meeting	➤ Pre-clinical and clinical study updates
05-05-2016	External Advisory Board meeting by web	➤ Pre-clinical and clinical updates and prelim. data
06-01-2016	Monthly Web-Meeting	➤ Recap of EAB meeting and suggestions, Pre-clinical and clinical study updates
07-13-2016	Monthly Web-meeting	➤ Pre-clinical and clinical study updates
08-03-2016	Web-meeting	➤ Pre-clinical and clinical study updates
08-25-2016	Immune genetics working group meeting	➤ Shipping samples, data update
09-07-2016	Web-meeting	➤ Clinical and pre-clinical study updates
10-05-2016	Clinical working group meeting	➤ Inclusion and exclusion criteria, recruitment update
10-05-2016	Web-meeting	➤ Pre-clinical and clinical study updates

Table 3. Consortium Working Groups

Working Group	Tasks	Members
Data Management Service Group	Assist with QC issues, data cleaning, data management and sharing, website management.	Christine Chaisson, DCC Consortium PI, co-PIs
Statistics Service Group	Perform analyses and provides statistical planning and advice for study investigators and research site PIs.	Timothy Heeren, Christine Chaisson, Consortium PI/co-PIs
Translational Working Group	Forum for Intellectual property and material (IP) issues, translation of results into papers, abstracts, new grant submissions and how clinical and preclinical results can inform each other.	Michael Pratt – BU Tech Transfer office Consortium PI, co-PIs Research site PIs, RIO

Behavioral Studies Working Group	Plan imaging protocols and provide quality control for multiple imaging sites. Plan behavioral testing protocols and coordinate preclinical and clinical studies for comparability.	Drs. Sullivan, Killiany, Kregel, Toomey, Steele, Klimas, Collier, Hutchinson, Maier, Watkins
Histopathology Working Group	Plan tissue studies of proinflammatory, glial, axonal transport and mitochondrial markers in similarly dosed animal and cell models.	Drs. Baas, Black, O'Callaghan, Fields, Maier, Watkins
Immune Genetics Working Group	Plan and implement studies assessing brain-immune interactions involving glia and proinflammatory cytokines/chemokines through genetic SNPs and mRNA and miRNA protein studies.	Drs. Collier, Hutchinson, Klimas, Steele, Sullivan, Watkins, Maier
Gulf War Veterans Advisory working Group	Update fellow GW veterans about GWIC research efforts and results, assist with recruitment efforts by making fellow vets aware of GWIC studies.	Denise Nichols, Frances Perez Wilhite, Lynn Santosuosso, Tim Demers, Christine Tron

Task 2a. BUSPH Data Coordinating Center (DCC) will create website, data collection forms, specimen tracking system and databases for the entire consortium including all preclinical and clinical sites.

Consortium website (<http://sites.bu.edu/gwic/>) is finalized and approval was obtained from each institution to use their logos on the site. Electronic data collection forms using REDCap software and CATI recruitment software are finalized and in use for subject screening and data collection. The study is utilizing Frontier Science's LDMS specimen tracking system for shipping samples to collaborating sites and for biorepository tracking. Training for the specimen tracking system has also been completed by all necessary staff. A refresher REDCap training was completed for all clinical staff and is in use as subjects are recruited and complete online questionnaires and screened for eligibility through the CATI system.

Task 2b. Develop manuals for the neuropsychological testing protocol, imaging protocols, specimen collection protocols and recruitment.

All cognitive administration and scoring manuals, specimen collection protocols have been finalized. All clinical staff has been trained to ensure proper quality control measures are in place for the clinical studies. This has been followed up by videotaping practice testing to ensure tester drift is not occurring and weekly phone calls are being conducted with testing staff as subject recruitment gets underway to answer any questions or discuss problems with test administration/scoring issues. This has proven helpful to ensure consistent test administration and scoring at all study sites.

Task 2c. Train researchers and staff on protocols and quality control measures for the clinical and preclinical studies

Training for researchers and clinical staff was completed at in-person meeting in Boston in August 2014 and continued to be monitored as described above. Working groups have finalized training procedures and protocols for cognitive, neuroimaging and laboratory procedures that are currently being used. As new staff members are added, they are trained appropriately.

Task 2d. Obtain stored blood samples from Hines VA study and send to Miami VA for analysis.

As described in Task 1, the Hines VA will draft a revision of the DUA to reflect the new arrangement that will be reviewed by GWIC investigators before submission for final approval. Once Hines can apply the GWIC algorithm for the Kansas definition of GWI, they can begin sending blood samples to Dr. Klimas at the

Miami VA. Dr. Klimas will perform cytokine analysis and send the results to Hines VA. Hines will conduct the statistical analyses. As soon as the DUA is approved, the blood samples will be sent to Dr. Klimas' lab at the Miami VA for analysis.

TASK 3. PREPARATION FOR CONSORTIUM PRECLINICAL STUDIES (MONTHS 9 - 24)

Monthly web meeting and working group meetings were ongoing during the past year to prepare for the planned preclinical studies and to coordinate overlap of the studies and to ensure that the same neurotoxicant dosing and exposure model of GWI was being used among the four preclinical study sites. The CDC site was tasked with comparing the mouse and rat models of GWI to ensure comparability for planned studies and to distribute dosed animals and animal tissue to the preclinical sites. The planning stage was successful as discussions progressed and initial studies began at the preclinical study sites. Preparation for the specific preclinical study sites are detailed below.

Task 3a. Prepare rat dosing models at CDC and distribute to other sites at NIH, Drexel, Temple and U-Colorado for planned studies of axonal transport, myelin integrity and learning and pain assessments.

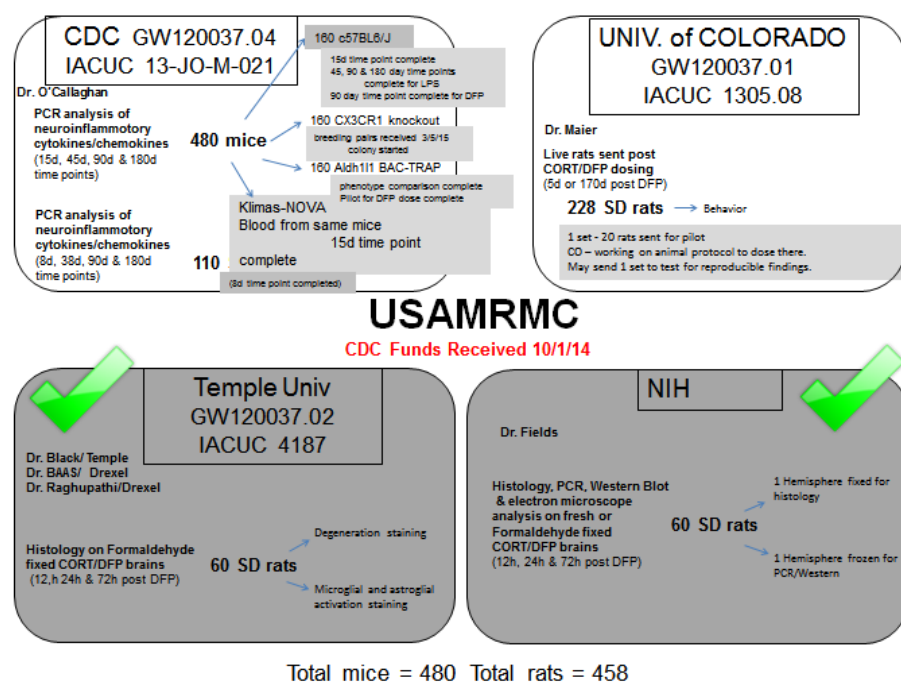


Figure 3. Distribution schematic of animals from CDC

- Dr. O'Callaghan at the CDC site prepared and validated rat dosing models based on his initial mouse GWI dosing models from prior DOD funded studies using chronic daily corticosterone (CORT) and 1 dosage of the sarin-surrogate DFP (O'Callaghan et al., 2015).
- An initial set of 20 rats [control group (n= 5), CORT only exposed group (n= 5), acute DFP exposed group (n= 5), and chronic CORT and DFP group (n= 5)] were dosed and sent to the University of Colorado to initiate behavioral studies with the rat GWI dosing model (once a memorandum of understanding was approved between CDC and University of Colorado to ship the animals). Animals in CORT groups were exposed to 200mg/L in their drinking water for 4 days followed by a single s.c. injection of saline (CORT group) or 1.5mg/kg DFP (CORT and DFP). Rats in the control and DFP

groups were given standard tap water for 4 days followed by a single s.c. injection of saline (Control) or 1.5mg/kg DFP (DFP). Dr. O'Callaghan also met with U-Colorado investigators to discuss having them dose unexposed animals at their site as well.

- The brains of fifty-five rats were removed and one hemisphere was flash frozen in dry ice and the other hemisphere was preserved in 10% formalin. CDC investigators sent brains from rats in the control group (n= 5), CORT only exposed group (n= 5), acute DFP exposed group (n= 5), and chronic CORT and DFP group (n= 5) to NIH. Three different cohorts of animals were exposed to their respective group conditions and separate sacrifices were performed at 12, 24, and 72 hours post DFP exposure.
- In the summer, CDC sacrificed 20 adult male rats [control group (n= 5), CORT only exposed group (n= 5), acute DFP exposed group (n= 5), and chronic CORT and DFP group (n= 5)]. Animals in CORT groups were exposed to 200mg/L in their drinking water for 4 days followed by a single s.c. injection of saline (CORT group) or 1.5mg/kg DFP (CORT and DFP). Rats in the control and DFP groups were given standard tap water for 4 days followed by a single s.c. injection of saline (Control) or 1.5mg/kg DFP (DFP). Rats were perfused and brains were preserved in 10% formalin brains. Perfused brains were then sent out to Dr. Killiany and Boston University collaborators for brains to be structurally imaged.
- ACUC amendment for dosing with DFP has been approved at University of Colorado and DFP rat dosing with subsequent behavioral and pain studies began being conducted at that location. In this experiment, more animals than expected died from the exposures. This resulted in U-Colorado and CDC stopping further dosing until a meeting with vets from CDC and U-Colorado could be held. The meeting was held and U-Colorado has gotten approval to begin dosing at their site again using palliative care models recommended by CDC vet and ACUC chair. It was also determined that the weight of the animals was a likely issue with prior animals being 100g less heavy than this group. By the time this was determined, the protocols had expired and weight monitoring and further palliative care models post-dosing were recommended for the new protocol that was recently approved.
- CDC has conducted these palliative care studies with the DFP model for the C57 mice experiments and the ACUC has agreed to approve rat studies if rats are exposed to the same palliative care procedures that are administered to mice exposed to CORT and DFP.

Current progress

- CDC has exposed rats to the same conditions described above and has shipped flash frozen and formalin preserved brains of rats sacrificed at 7, 14 and 21 days post-DFP exposure to NIH collaborators. Animals in the CORT groups will be exposed to 200mg/L in their drinking water for 4 days followed by a single s.c. injection of saline (CORT group) or 1.5mg/kg DFP (CORT and DFP). Rats in the control and DFP groups will be given standard tap water for 4 days followed by a single s.c. injection of saline (Control) or 1.5mg/kg DFP (DFP). The 7, 14, and 21 day time points will allow for investigation of neuronal changes or degradation at further time points post condition exposure.

Task 3b. Develop co-cultures of rodent oligodendrocytes in cell culture chambers for electrical stimulation of axons and development of myelination in vitro at NIH.

Dr. Fields and Dutta have been making the cell cultures from embryonic mice and rats for studies of myelination and the co-cultures are currently developed in the cell culture chambers for the ongoing and planned oligodendrocyte and myelin studies.

1. An assay to quantify the effects of GW toxicants on the process of myelination in-vitro was developed. Cholinergic neurons are being myelinated in co-culture with oligodendrocytes, providing a framework to study the effects of GW agents on axonal myelination by oligodendrocytes. Techniques are being developed to produce purified motor neuron cultures. OPCs have now been

combined with these neuronal cultures in multicompartiment chambers for electrical stimulation of axons and treatment with pharmacological agents.

2. GW agents primarily disrupt cholinergic neurotransmission. Like neurons, oligodendrocytes express several muscarinic receptors, types 1-5, and our preliminary research shows that oligodendroglia can respond to cholinergic stimulation. We have acquired and housed muscarinic receptor 1-5 KO mice, from which oligodendrocytes can be isolated and cultured with GW agents, so as to definitively delineate the role of these agents on oligodendrocyte biology and development. NIH investigators have also received brain tissue from CDC to perform histopathology studies of myelin from exposed animals. The brains from rats exposed to neurotoxins by Dr. O'Callaghan's lab were analyzed for changes in myelin and other proteins by western blot. This included 60 samples (brains), 12 treatment conditions, 3 time points, (12, 24 and 72 hr) for the proteins olig 2, MBP, and GAP-43. The results show that myelin proteins are affected, but the result is surprising. Rather than decreasing, as would be expected with myelin damage, we find an increase in myelin basic protein (MBP) levels increase significantly 72 hrs after treatment. This result, however, shows that myelinating glia are being affected by the treatments modeling exposures that patients with GWI are likely to have experienced. This is a very important finding with respect to the research on white matter damage found in GWI. We suspect that the increase in MBP at this time point may reflect an adaptive response to the toxins in an attempt to recover from white matter injury. Histological analysis of the same brains will allow us to investigate this. A staff member was hired to do the microtome sectioning of these brains and immunocytochemical staining. This work is well underway and histological and immunocytochemical analysis will be shown in future updates. Dr. Field's lab also investigated levels of olig2, a transcription factor for oligodendrocytes, to determine if oligodendrocytes are dying or increasing in number after treatment. No change in this protein was observed. Dr. Field's lab also tested for changes in GAP-43, because this protein is a marker for axon sprouting. One possibility for an increase in MBP after treatment might have been that axons were damaged and had begun to sprout in recovery. These sprouts would then need to be myelinated, which could account for the increase in MBP protein we found after treatment. This hypothesis was not supported. GAP-43 levels are not different among treatment groups. Interestingly, the only treatment condition where MBP levels changed was in the CORT+DFP group. Neither DFP nor CORT alone caused a change. This result is consistent with other results that Dr. O'Callaghan has reported at CDC (O'Callaghan et al., 2015).

The next step was to extend the treatment conditions beyond 72 hours and analyze MBP levels, because myelination is a long-term process and the shorter time points may not have captured the full response of what is occurring. Following the recommendation of the EAB, the lab has advanced the experimental time-line by ceasing work on the first aim to instead investigate the last aim concerning in vivo studies. The lab felt it was important to determine if the GWI animal model that Dr. O'Callaghan has developed results in any effects on myelin. That research identified MBP as being altered by the in vivo treatment. Based on that finding Dr. Fields planned longer-term treatments to study effects on myelin. In the results of these longer-term analyses, we saw that increase in MBP levels persisted for the CORT+DFP condition in the longer time-points in Dr. O'Callaghan's rats, as compared to controls. Moreover, unlike in earlier time-points (72 hours and less), MBP levels seem to increase modestly with CORT alone and DFP alone treatments too at 7 days and 21 days. We are trying to find a biological basis for why this would be so. How CORT and DFP treatment, either alone or in combination, can influence oligodendrocyte development in such a way so as to cause increase in MBP expression.

Returning to the earlier aims identifying the cholinergic receptors on oligodendrocytes by using calcium imaging. This work shows a rich array of cholinergic receptors are active in these cells and thus they would be affected by exposure to nerve agents. The lab now needs to determine how these cells are affected.

An important second component of O'Callaghan's in vivo model of GWI is prior exposure to corticosterone. Corticosterone has complex cellular and systematic effects, which make it difficult at this point to fully understand its mechanism of action in the GWI model. Dr. Fields therefore began testing oligodendrocytes in cell culture and found that these cells do respond to corticosterone treatment by undergoing a sharp rise in intracellular calcium. He is finding complex interactions between corticosterone and cholinergic receptor activation, which can be additive, synergistic, or antagonistic to the rise in intracellular calcium caused by cholinergic receptor activation.

Finally, the lab began the co-culture experiments with dorsal root ganglion neurons and oligodendrocytes to determine whether cholinergic receptor activation and inhibition have an effect on myelination. The focus of these experiments at the present time is to develop and optimize this preparation for studies on cholinergic signaling in myelination.

The lab has developed and performed a preliminary study on the effect of Acetylcholine stimulation on myelination in a co-culture system of oligodendrocytes and DRG neurons. The aim is to assess how short term and long term treatment of Acetylcholine will impact myelination of axons in a co-culture system. Acetylcholine was either added short term (3 days and then withdrawn) or long-term (throughout the study, 17 days). Cultures were grown for 17 days and then fixed and stained for MBP and Olig2.

TASK 4. PERFORM PRECLINICAL CELL AND ANIMAL STUDIES (MONTHS 9-42)

The preclinical studies started later than initially anticipated for the CDC site and those sites waiting for distribution of animals or tissue from the CDC site because an interagency agreement for the release of funds from DOD to CDC took approximately 8 months out of the first year of funding to be released. Considering this delay in funding, a significant amount of work has been initiated at the 5 preclinical study sites to date. Specific progress to date is listed below for each of the sub-studies.

Task 4a. Assess for axonal transport integrity in rodent and cell models exposed to either GW-relevant neurotoxins or cytokines (Drexel - 30 Sprague Dawley rats, Temple - 27 Sprague Dawley rats).

Items completed to date for the axonal transport and microtubule integrity group include: 1) An enhanced mitochondrial transport assay using a CMXRos mitochondrial dye which is sensitive to mitochondrial membrane potential, thus enabling us to not only appreciate mitochondrial transport but also the oxidative stress of the neuron. Preliminary experiments using this new dye are underway. Results from our previous mitotracker experiments suggest a bidirectional inhibition of mitochondrial transport after treatment with DFP.

2) CAMSAP2 experiments are complete and the protocol for CAMSAP2 is currently included in a paper under review at the Journal of Cell Biology. This technique is now being used as an additional outcome measure of how microtubule integrity is affected by Gulf War neurotoxins, as CAMSAP2 stabilizes the minus-ends of microtubules.

4) CORT and DFP working concentrations have been established and all assays have been optimized.

5) Studies using CORT + DFP experiments are underway. In these experiments, mature cortical and hippocampal neurons are pretreated with CORT prior to application of DFP at five concentrations (0.1 nM, 1 nM, 10 nM, 100 nM, and 1 μ M). Live-cell imaging of mitochondrial transport, microtubule polymerization, and microtubule transport will be performed. A parallel set of experiments will be fixed and stained for acetylated tubulin and total tubulin. We will analyze the ratio of acetylated (stable microtubules): total microtubule mass contained in neurons in vitro in control and treatment groups to better understand the loss of microtubule mass observed in previous experiments.

- 6) Our next step is to isolate and culture rat cortical astrocytes. Once cultures are established, astrocytes will be primed with CORT and treated with DFP. The culture supernatant will be collected and applied to purified cortical and hippocampal neurons to explore how cytokines secreted by astrocytes affect neurons in vitro. Parallel experiments will assess the cytokine profile under control and treatment conditions.
- 7) Histological studies on the in vivo rodent model reveal no obvious changes in total tubulin or acetylated tubulin in the brain compared to controls at an acute time point. This was expected as we previously observed no obvious signs of cell death or impaired axonal transport, which is consistent with toxin dosage that accurately reflects that to which the soldiers were exposed. Experiments will investigate the aforementioned parameters at chronic time points. In these experiments, immunostaining for microtubules (total and acetylated tubulin), axonal transport defects, and cell death will be explored.
- 8) Dr. Liang (Oscar) Qiang has arrived to Drexel University where he will use neurons differentiated from GW veteran-derived induced pluripotent stem cells (see Figure 3 below) as part of a funded New Investigator proposal to Dr. Peter Baas in collaboration with Dr. Sullivan (GW140086). A review paper on the hiPSC cells has been submitted to Neurology for publication.
- 9.) Mitochondrial transport figures are being finalized for publication. Replication experiments have been run.
- 10.) Western blot data for human and rat samples are ready for publication.
- 11.) Experiments using potential treatments (i.e. tubacin, NAP peptide, etc.) are planned and have been started for tubacin treatment, others are still planned.
- 12.) Investigators have begun a collaborative experiment with Dr. Rodrigo Espana to explore the amount of released neurotransmitters in the presence of DFP or CORT + DFP.
- 13.) A review article has been submitted for review and a primary research article is in preparation for submission for publication.
- 14.) A Complete data workup and figure preparation for HPLC are planned for the next quarter, as well as the completion of western blot experiments.

The funded iPSC work is well connected to the consortium objectives as it is designed to assess axonal transport and microtubule dysfunction in GW relevant exposures including DFP and cortisol (human equivalent of CORT). The hypothesis is that exposure of neurons and/or neuroinflammatory cells to GW toxins caused long-lasting axonal transport/microtubule defects in neurons, and that these defects lead to a loss of microtubule mass, a change in the proportions of stable and labile microtubule mass, and/or flaws in the lattice of the microtubule that lead to abnormalities in how molecular motor proteins and other microtubule-related proteins interact with the microtubule and move down the axon. IRB and HRPO approval has been obtained for the stem cell study with GW veterans, recruitment is well underway, the first batch of hiPSCs have been reprogrammed and a position paper has been submitted to Neurology for review.

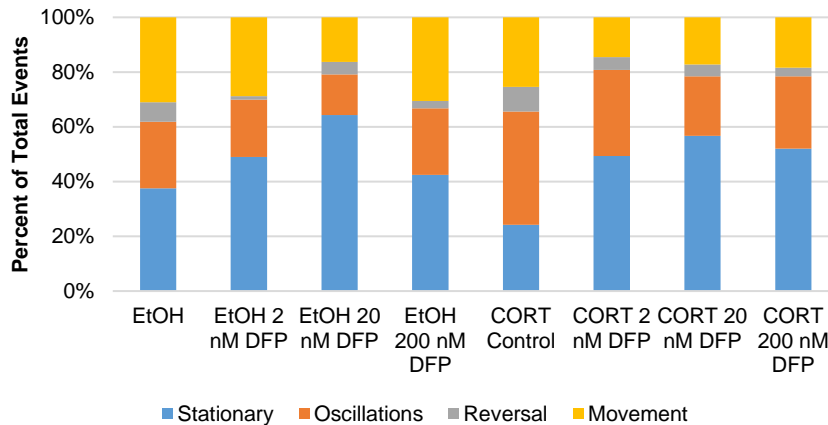


Figure 1 – DFP causes a concentration-dependent increase in stationary mitochondria

Rat cortical neurons were pre-treated with either EtOH or CORT for 24h before a 24h treatment with three different concentrations of DFP. Movement of mitochondria was observed using the mitochondrial stain Mitotracker and mitochondria behavior was binned into four different categories.

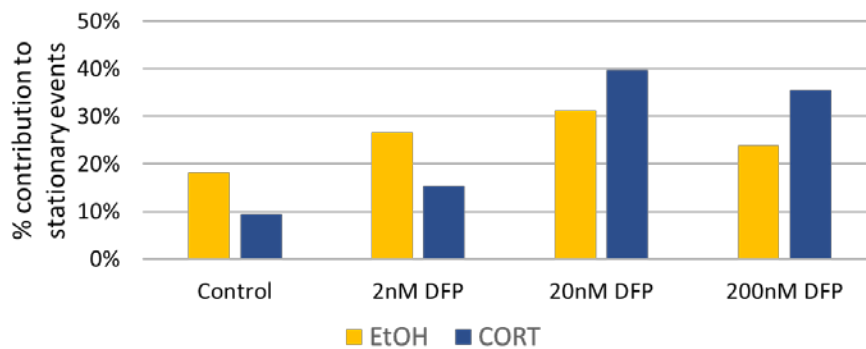


Figure 2 – CORT seems to enhance the effect of DFP on stationary events in mitochondrial transport.

Microtubule-based transport is essential for synaptic function and neurotransmitter release. Our preliminary work shows that prolonged stressors affect dopamine release and DFP after prolonged physical stressors significantly reduces the amount of extracellular dopamine in Figure 3 below.

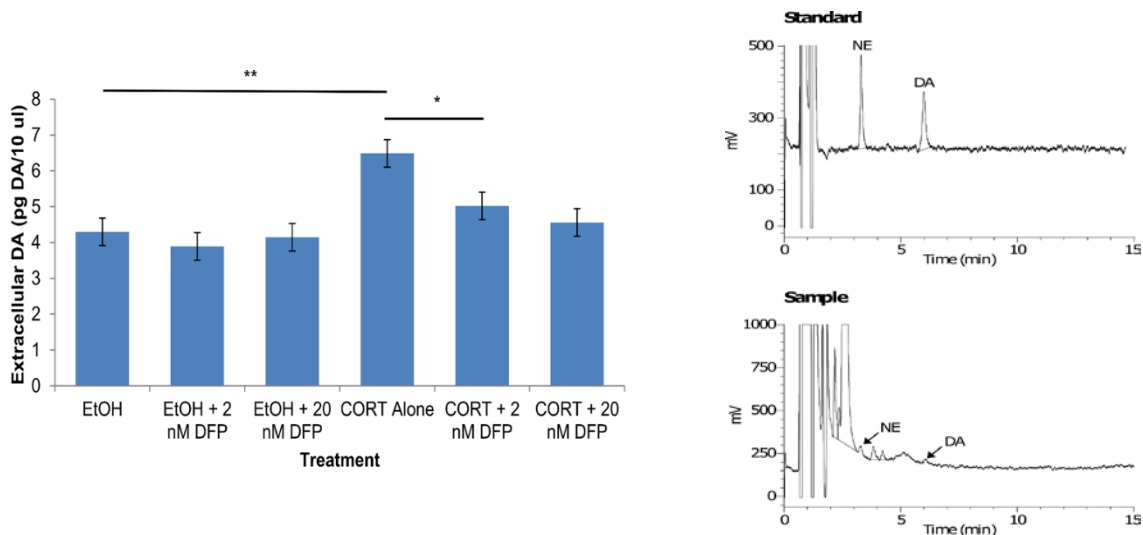


Figure 3 - Quantification of Extracellular Dopamine by HPLC

Treatment development has begun by assessing the role of tubacin on stabilizing microtubules and thus reducing acetylated microtubules. Using western blot analysis, Dr. Baas investigated the effect of DFP alone or DFP exposure after CORT pretreatment. Results showed that DFP reduced the stable fraction of microtubules (acetylated tubulin). Using tubacin, an FDA-approved HDAC6 inhibitor, changes after DFP exposure, with or without CORT pretreatment in Figure 4, were stabilized and restored to control levels. This indicates a potential clinical translational therapeutic strategy for GWI.

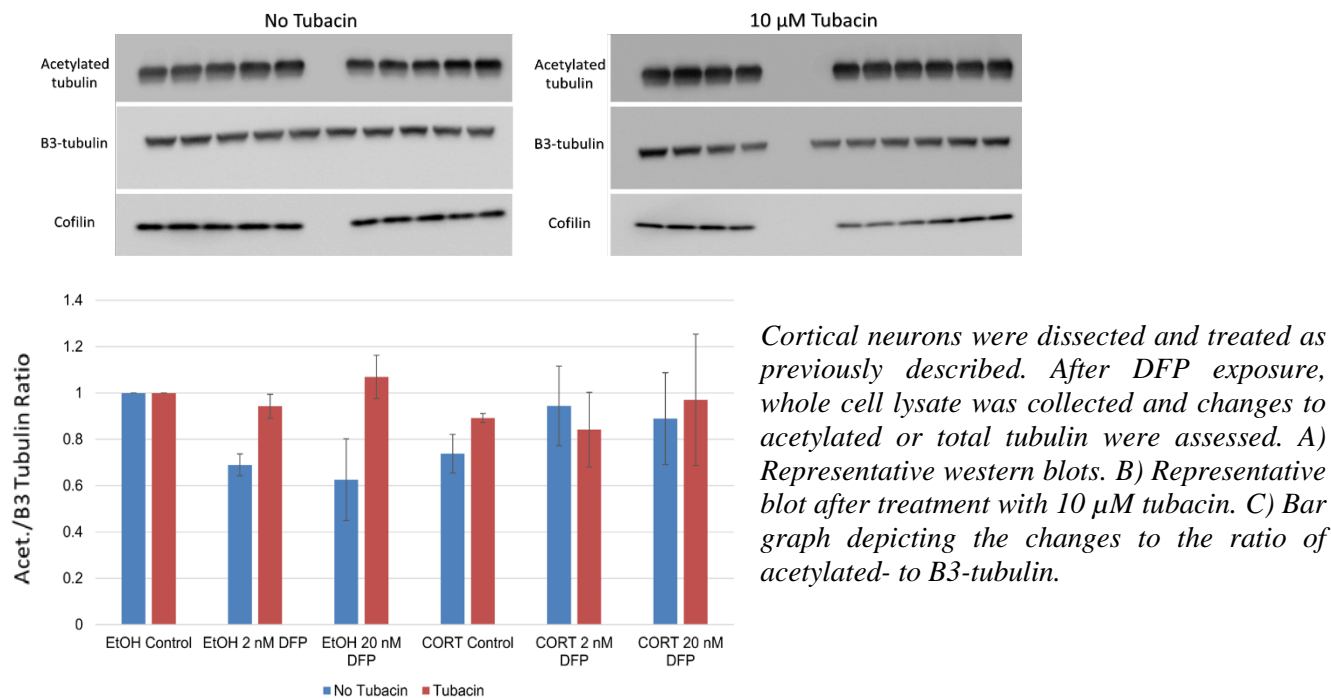
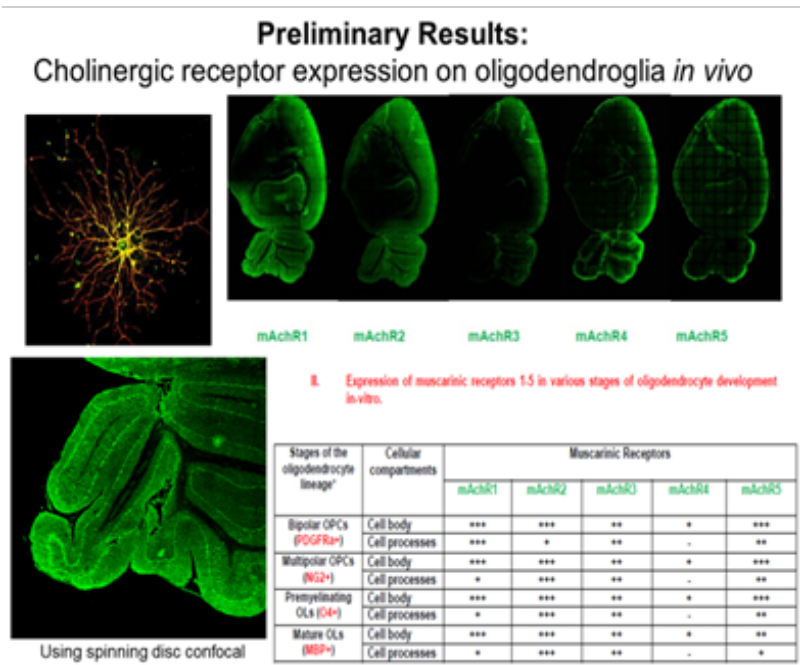


Figure 4. Tubacin treatment appears to improve stabilized microtubule ratio after CORT + DFP Task 4b. Assess for myelin integrity in rodent and cell models exposed to either GW-relevant neurotoxicants or cytokines (NIH – 624 NIH/S mice and 208 rats).

It has been determined that there are functional cholinergic receptors on oligodendrocytes and the site-PI Dr. Fields is characterizing the type of receptor and how they change during development, using immunocytochemistry and live cell calcium imaging.

1. We are characterizing the expression of muscarinic receptor type 1 through 5 in oligodendrocyte lineage cells in-vitro, in primary oligodendrocyte cultures and in co-culture with neurons. We are also characterizing their expression in the adult mouse brain in-vivo. We observed that in oligodendrocytes, these receptors are differentially expressed in a complex



manner, depending on differentiation stage of the cells, their location in the brain. Preliminary data also show that there are subcellular differences in receptor subtypes on oligodendroglial cells; for example on the cell processes vs. cell body. These initial data indicate that not all oligodendrocytes express these receptors in-vivo and the cells that do are closely associated with cholinergic projection systems in the brain.

2. We are testing functionality of these receptors via live-cell calcium imaging of primary oligodendrocytes in culture to assess their response to broad spectrum muscarinic receptor agonists, Acetylcholine and Carbachol, via a rapid Calcium influx, indicating that the receptors on these cells are functional.

Future work will include characterizing the function of muscarinic receptors on oligodendrocyte biology and development, so as to provide a framework for future studies with GW agents. Once we have identified the expression of cholinergic receptors on oligodendrocytes we will determine their function in biological processes that could account for white matter injury seen in GWI. This includes effects on cell proliferation, survival, differentiation, formation and maintenance of myelin. Bioassays for all of these functional effects will be undertaken. After determining the cellular effects in culture (e.g., proliferation, survival, differentiation, migration, myelination, demyelination) this information will inform us how best to undertake animal studies and the best strategy to measure the effects. Our immediate goals are to determine how disrupting Ach signaling (and neuroinflammation/cytokines) could cause the white matter deficits seen in GWI. There are two general ways that this could happen: 1) Direct effects on oligodendroglia, 2) alternatively, by disrupting cholinergic signaling (AChE, BChE) from axons that affect oligodendrocytes and myelination. The use of experiments on OPCs in monoculture vs co-culture with neurons will enable us to make this critical distinction.

Researchers have discovered that release of synaptic vesicles from perinodal astrocytes remodels myelin structure and exposes the node of Ranvier to disruption. This is relevant to immune attack on myelin and would be one of the consequences of GFAP upregulation that accompanies TBI and neurotoxicity. This has led to the identification of a possible biomarker for myelin damage (neurofascin 155) that the GWIC investigators will now investigate in CSF and serum samples of veterans with GWI from the consortium biorepository as part of a newly funded study in collaboration with Dr. Sullivan, Klimas, Krengel and Duke University investigators (GW140140). Dr. Fields is developing methods with high sensitivity to detect this protein fragment (ELISA methods) for further study and progress to date is listed below.

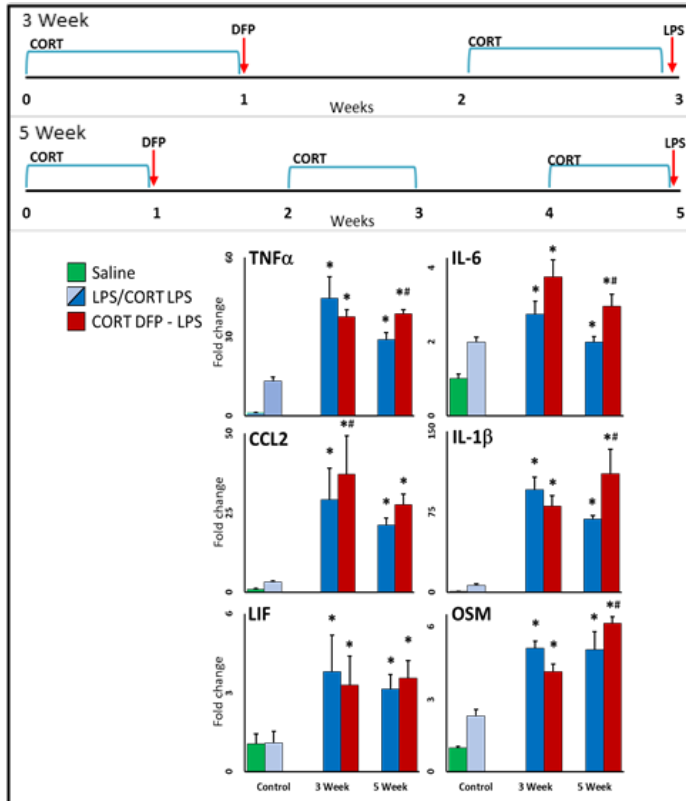
1. Dr. Fields lab has developed a custom Sandwich-ELISA that can potentially detect cleaved fragments of Neurofascin 155. The lab has received the custom antibodies and are beginning to characterize the efficacy of the ELISA system.
2. Future work will include testing the NF155 ELISA kit for efficacy and if everything works out, we can use the assay to look for cleaved NF155 fragments in CSF and serum samples of veterans with GWI (from the GW repository in the currently funded study, GW140140).

Task 4c. Assess whether persistent priming of neuroinflammation occurs chronically with GW-relevant neurotoxins and intermittent corticosterone exposure to model the chronic nature of GWI (CDC – 100 C57BL/6 mice).

- CDC investigators performed cytokine expression profiling in the rat after DFP and CORT+DFP and determined that the enhanced cytokine expression seen in the GWI mouse model could also be generalized to the rat strain used in Dr. Maier's studies at the University of Colorado.
- Results from a July 2015 study using an LPS challenge at 21 days post-exposure to 4 days of CORT + DFP, 10 days off, and another 4 days of CORT produces enhanced neuroinflammation, and some markers trend toward greater neuroinflammatory levels for mice initially exposed to CORT + DFP.

- Data at even later time points 90 and 97 day post-DFP dosing experiments (approximately 10 human years after return from theater) with intermittent 4 day CORT re-exposures (stress mimic) and an LPS challenge (inflammatory insult) at 90 days were collected on September 2015. Results from this study revealed that 1) at least 4 day CORT exposures just prior to exposure to an insult or inflammagen (e.g., LPS) are necessary for augmented neuroinflammation. Later time points after initial DFP and CORT exposure that do not have both CORT treatment and an exposure to an insult (e.g., LPS) will not result in enhanced neuroinflammation. 2) CORT DFP exposure initially increases inflammation but this inflammation abates over time, and increased neuroinflammation is not seen without initial exposure to the combination of high levels of physiological stress and a future exposure to insult or injury. 3) While CORT priming and initial DFP exposure and intermittent 4 day CORT exposure produces enhanced neuroinflammation at later time points (e.g., 90 days) after exposure, these inflammation levels were less than the inflammation levels we saw in results from our preliminary studies using 7 day increments of CORT exposure. In these past studies, exposures of CORT in 7 day increments for 90 days produces a greatly augmented neuroinflammation to an LPS exposure.
- In the past, CDC conducted a potential treatment study to examine efficacy of minocycline administration post-dosing as the effect of minocycline on neuroinflammation in this model had only been investigated as a pre-exposure treatment (O'Callaghan et al., 2015). Mice were exposed to CORT (200mg/L) for 4 days followed by DFP (4.0mg/kg, i.p) and minocycline prior to LPS insult (0.5mg/kg, s.c.) on day 7. Results from this study revealed that CORT DFP exposure produced augmented neuroinflammation greater than CORT alone to an LPS challenge. Furthermore, treatment with minocycline 1 hour prior to LPS exposure significantly reduced neuroinflammation in most markers in our panel. Specifically, minocycline treatment removed the enhanced neuroinflammation instigated by DFP, returning most inflammatory cytokine levels to that of CORT LPS.
- Comparison of different CORT exposure and neuroinflammatory effects at later time points is important in establishing the correct CORT exposure paradigm and LPS dosing of mice in the GWI model. CDC comparisons revealed that 7 days of CORT exposure on and off prior to an LPS insult appears to be the most effective at inducing neuroinflammation. Additionally, LPS dosing at 2.0mg/kg induced a greater increase in pro-inflammatory cytokines, and these increases seemed to hit a ceiling especially in the CORT exposed groups. Thus, the 0.5mg/kg dose of LPS seems to be a more sensitive measure of neuroinflammation when examining group differences in this paradigm.
- ALDH1L1 BAC-TRAP mice have been used for initial studies with the CDC GWI exposure protocol, and we see that DFP exposure produces a similar phenotype in these animals to that seen in C57 exposed animals. The TRAP procedure was then used to isolate actively translating mRNA from astrocytes (ALDH1L1-containing cells) at 6 hours after DFP exposure with and without CORT. Analysis of this data is ongoing. Preliminary data supports the use of this model to understand the enrichment of GWI-relevant molecular signatures and signaling pathways in astrocytes over total tissue expression (mixed cell population), as 110 and 211 significantly altered genes in cortical astrocytes were found to be expressed 10- and 5-fold over total cortex, respectively. Initial functional analysis of the set of 211 genes indicates that these genes are largely involved in immune signaling and show particular enrichment for cytokine and complement factor signaling.
- An animal protocol to complete the 3, 4, 5 week GWI paradigms in the ALDH1L1 BAC-TRAP animals has been approved and animals are being bred to complete this study.
- Initial investigation of neuroinflammatory responses in our CX3CR1 knock out mice was conducted using the bacterial mimic and known inflammagen, LPS. These mice had pronounced inflammatory reactions to low dose LPS exposure (0.5mg/kg), and following 4 days of CORT exposure.

- CDC has performed a comparative study of 3 and 5 week long GWI “phenotype” exposure paradigms where mice receive 7 days of CORT every other week for the specified time. In addition to providing more points for intervention (i.e. 3 intervening weeks between CORT DFP, and CORT LPS exposures), the 5 week study demonstrated a more consistent trend of greater inflammatory
- expression in groups exposed to CORT DFP LPS over CORT LPS, compared to the 3 week cohort.

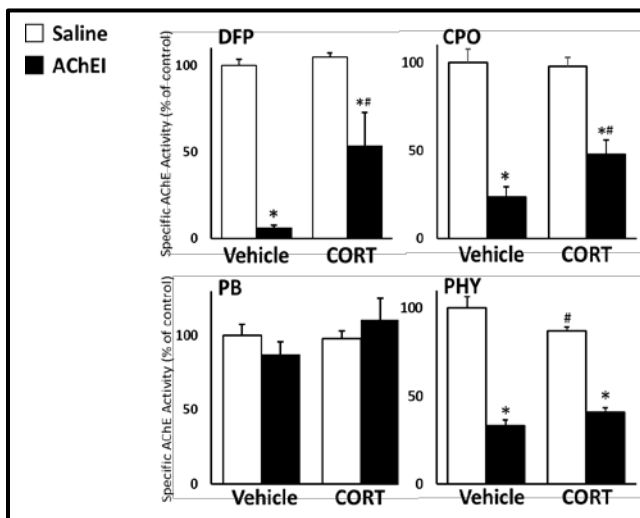


Thus, the 5 week paradigm will be used for screening potential treatments.

- CDC has performed preliminary dosing studies with propranolol to identify the appropriate dose to be used in subsequent treatment studies. Once an appropriate dose has been established, CDC will use this dose of propranolol in our 5 week GWI model paradigm to screen for the effectiveness of this treatment. If this compound is successful in reducing enhanced neuroinflammation that mice exposed to the GWI paradigm display, further tests will be conducted to see if multiple doses of this drug at different times during the paradigm should be administered in order to better reduce symptoms of GWI.

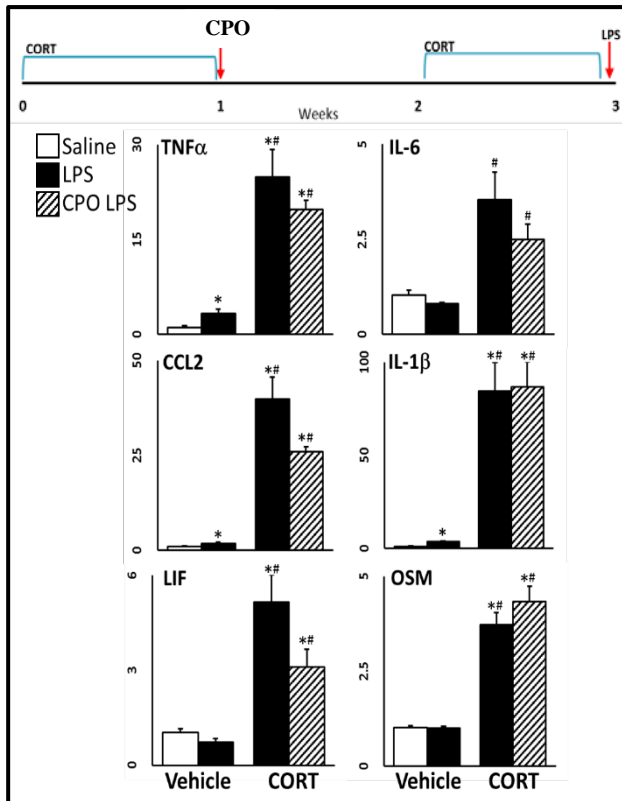
- In accordance with previously discussed acetylcholinesterase (AChE) activity assays presented to the EAB, CDC is currently performing mass spectrometry analysis of acetylcholine levels in DFP and CORT DFP treated

mice to provide a comprehensive analysis of the cholinergic effects of DFP in the brain.



nor does it result in any neuroinflammation (PHY alone or CORT PHY).

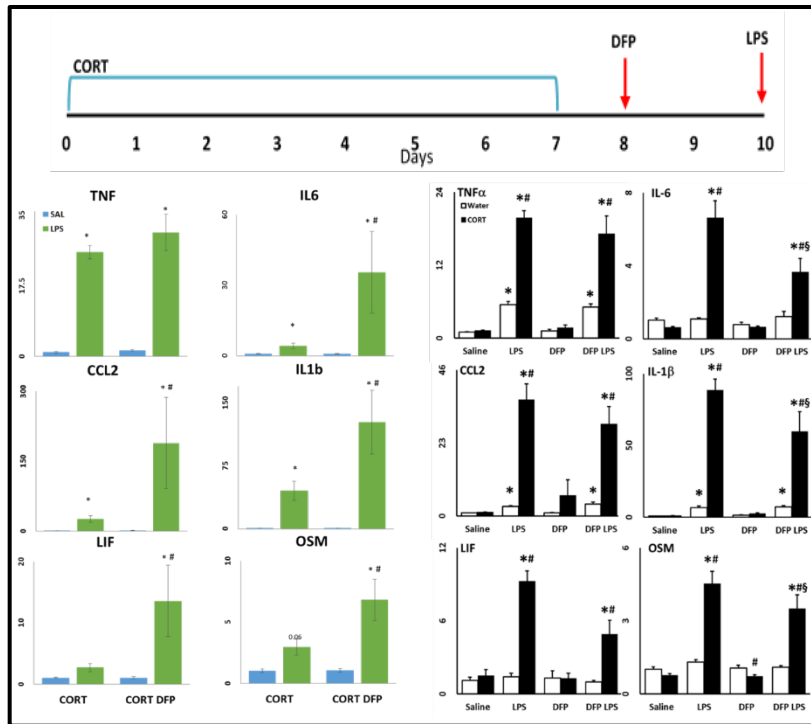
From the AChE activity assays, there does not seem to be a correlation between enzyme inhibition in the brain and neuroinflammation. While DFP and chlorpyrifos oxon (CPO) both irreversibly inhibit AChE, pretreatment with CORT significantly reduces the level of inhibition caused by these agents. This is in contrast to neuroinflammatory endpoints which show increased expression with CORT pretreatment. We also find no evidence of neuroinflammation with the reversible prophylactic, pyridostigmine bromide (PB), nor any indication that prior stress hormone exposure (CORT) changes the permeability of PB to the brain (see AChE activity). Furthermore, using the brain-penetrant reversible AChE inhibitor, physostigmine (PHY), we find that CORT does not alleviate the enzyme inhibition caused by this drug



- CDC has performed a preliminary short-term, 3 week exposure paradigm expanding the GWI phenotype to include chlorpyrifos oxon (CPO; pesticide). Mice were exposed to CORT (200 mg/L) in the drinking water for 7 days followed by an exposure to CPO (2 mg/kg, i.p.). The animals were then exposed to CORT every other week for a total of 3 weeks. Animals were then challenged with LPS (0.5 mg/kg, s.c.) 6 hours before sacrifice. The results for the 3 week paradigm using CPO are similar to the result for DFP in a similar paradigm (see figure above). The results from this study will be expanded upon to evaluate the same dose at 5 weeks, as well as testing a higher dose of CPO in similar paradigms.

Task 4d. Assess the relative contributions of astrocytes and microglia in rodent GWI neuroinflammatory models in order to identify which glial markers will provide the best candidate “drugable” targets (CDC 40 C57BL/6 mice; 40 ALDH1L1 mice; 40 B6.129-Cx3CR1 mice).

- ALDH1L1 BAC-TRAP mice have been used for initial studies with the CDC GWI exposure protocol, and we see that DFP exposure produces a similar phenotype in these animals to that seen in C57 exposed animals. The TRAP procedure was then used to isolate actively translating mRNA from astrocytes (ALDH1L1-containing cells) at 6 hours after DFP exposure with and without CORT. Analysis of this data is ongoing. Preliminary data supports the use of this model to understand the enrichment of GWI-relevant molecular signatures and signaling pathways in astrocytes over total tissue expression (mixed cell population), as 110 and 211 significantly altered genes in cortical astrocytes were found to be expressed 10- and 5-fold over total cortex, respectively. Initial functional analysis of the set of 211 genes indicates that these genes are largely involved in immune signaling and show particular enrichment for cytokine and complement factor signaling.
- An animal protocol to complete the 3, 4, 5 week GWI paradigms in the ALDH1L1 BAC-TRAP animals has been approved and animals are being bred to complete this study.
- Initial investigation of neuroinflammatory responses in our CX3CR1 knock out mice was conducted using the bacterial mimic and known inflammagen, LPS. These mice had pronounced inflammatory reactions to low dose LPS exposure (0.5mg/kg), and following 4 days of CORT exposure.

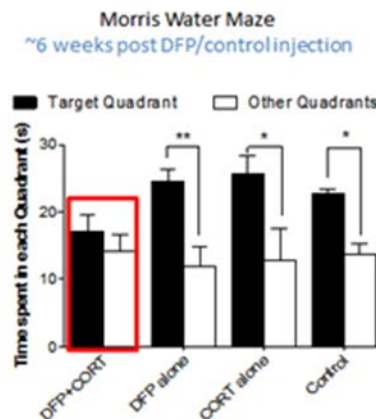


• CDC has performed a preliminary experiment exposing CX3CR1 KO mice to a short term GWI paradigm. These animals were exposed to 200 mg/L CORT in the drinking water for 7 days followed by a single injection of DFP at 4 mg/kg, i.p. Two days later, the mice were exposed to a single, s.c. injection of 0.5 mg/kg LPS to elicit an inflammatory response. Elimination of CX3CR1 prevented the priming of CORT DFP exposures on the subsequent LPS response (see figure below as compared to treatment in C57BL/6 mice (left panels)).

4e. Assess the relationship between behavioral testing of learning and memory and enhanced pain, in rodent GWI neuroinflammatory models by assessing hippocampal functioning with a fear conditioning task (U-Colorado- 120 rats).

The purpose of this research was to determine whether the combination of exposure to corticosterone (CORT; mimicking “stress”) and DFP (mimicking sarin organophosphate exposure) produces cognitive deficits in rats and whether any such deficits are hippocampal in origin. To this end, Dr. O’Callaghan prepared 4 groups of rats for an initial study—Control, DFP, CORT, and DFP + CORT. For this initial study 6 mice were included in each group, and were shipped to University of Colorado after dosing at CDC. These rats were tested for the formation of spatial memory (hippocampal dependent) using the Morris water maze and for contextual fear memory after fear conditioning. Anxiety was also assessed as indicated by juvenile social interaction (JSE) ratings. The water maze data were striking (see figures below). Acquisition to escape by finding the safe platform in the spatial version of this task was severely impaired the DFP + CORT group. However, escape was acquired. We then tested for memory of where the escape platform is located by testing the animals 24 hrs later with the escape platform absent. If the subject remembers they are expected to spend their time in the quadrant where the platform had been located, the target quadrant was Q2,

CORT + DFP impairs long-term memory



in

and all the groups except the DFP + CORT group spent much more time in this quadrant than would be expected by chance. That is, they remembered where the platform was located. The DFP + CORT group showed worse memory performance. The fear conditioning data were less clear and more subjects are needed in further studies to make more definite conclusions. These data are highly encouraging and larger group sizes will make results and conclusions more evident. One difficulty encountered was that JSE testing suggested that all animal groups were anxious. This may have been related to the fact that the animals were shipped from Dr. O'Callaghan's laboratory after dosing treatment, and so plans were set to be able to do the "dosing" directly in Colorado under Dr. O'Callaghan training as well as at the CDC laboratory for future planned studies. As described in Task 3, IACUC protocols were submitted for memory and pain studies at U-Colorado to dose in-house and initial pilot studies resulted in more animal deaths than expected. Studies were stopped until it could be determined how to improve animal survival post-dosing. This was determined by using animals of less weight (300g vs 400g) and instituting palliative care models post-dosing. IACUC approvals are now in place again and after ACURO rewrite approval, studies will begin again.

Task 4f. Compare central and peripheral markers of neuroinflammation in brain tissue and blood samples from GWI neuroinflammatory rodent models (CDC – 60 mice, Nova).

This study planned to compare blood samples and brain tissue proteins from 60 dosed mice. The animals were dosed and sacrificed and the blood samples were sent to NOVA Southeastern university for analysis of proinflammatory cytokine/chemokine panels. Analyses have been completed and sent back to Dr. O'Callaghan's laboratory to be compared with brain tissue studies. Initial results from early time points did not show strong markers of peripheral inflammation in comparison to central markers of inflammation. Liver and serum samples were obtained from the GWI 15 day model cohort at CDC. Liver mRNA expression (CDC) and serum protein concentration (NOVA) was compared to mRNA brain data from the CDC for mice in the GW model: control group (n= 5), CORT only exposed group (n= 5), acute DFP exposed group (n= 5), and chronic CORT and DFP group (n= 5). While CORT pretreatment exacerbated DFP-induced neuroinflammation, in the periphery the opposite effect occurred with CORT pretreatment ameliorating DFP-induced proinflammatory cytokine expression in the liver and serum. The divergence of expression of pro-inflammatory cytokines seen in brain and in the periphery suggests that the initial symptoms of GWI may be a result of the effects of CORT and DFP in the brain only or a compensatory mechanism in the periphery.

CDC has now sent mouse serum samples to NOVA Southeastern University for cytokine analysis following acute exposure to DFP, as well as following the 3-week and 3-month GWI phenotype paradigms. With the longer exposure periods, both brain and serum samples show a significant inflammatory priming in the CORT DFP exposed samples that are subsequently challenged with LPS. This analysis is ongoing and may suggest important similarities with the clinical studies.

Task 4g. Compare the effectiveness of several relevant preclinical treatments for GWI in cell and animal studies, including inflammatory glial activation modulators, antioxidants, and neuroprotective peptides (Drexel, Temple, CDC, U-Colorado)(20 animals per treatment).

These important experiments have begun and will continue as more information is known from the initial pathobiology studies in order to use those results to target appropriate choices for study. As previously mentioned, initial studies using minocycline and propranolol have been started by Dr. O'Callaghan's lab at CDC and additional studies using the neuroprotective peptide tubacin have been conducted at Drexel University by Dr. Baas' lab with promising initial results. In addition, Dr. Peter Grace at U-Colorado was awarded a New Investigator award to further test inflammatory glial modulators to reduce chronic pain in rat GWI models. The other study sites will begin treatment experiments shortly as well. On the clinical side, Drs. Toomey and Sullivan have obtained funding for a clinical treatment trial of d-cycloserine and Drs. Klimas,

Krengel and Sullivan have obtained funding for a phase III clinical trial of Co-Q10 in GWI. Thus, treatment development is occurring at the preclinical and clinical sites simultaneously.

TASK 5. SCREENING, RECRUITMENT AND ASSESSMENT OF GULF WAR VETERANS FROM THREE SITES (MONTHS 9-42)

Participant recruitment and screening is ongoing at the Boston and Miami sites. Currently 164 participants have been screened and 71 have been assessed at the Boston and Miami sites. The Texas site has received their local IRB and HRPO approvals and will begin recruitment shortly. See current recruitment table below. Although below initial estimates, recruitment is steadily increasing and retention is excellent from time of screening to completion of study (see figures below).

**GWIC: Gulf War Illness Consortium
Screening and Enrollment Summary Report
10/12/2016**

	Total	Boston	Miami	Texas
Number of Subjects Contacted	174	133	41	0
Number of Subjects Screened	164 (94.3%)	123 (92.5%)	41 (100.0%)	0 (%)
Number of Subjects Eligible	108 (65.9%)	70 (56.9%)	38 (92.7%)	0 (%)
Number of Subjects with Appointments Made	83 (76.9%)	47 (67.1%)	36 (94.7%)	0 (%)
Number of Subjects Assessed	71 (85.5%)	47 (100.0%)	24 (66.7%)	0 (%)

	Total		Boston		Miami		Texas	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Number of Subjects Screened	142	22	105	18	37	4	0	0
Number of Subjects Eligible	89	19	55	15	34	4	0	0
Number of Subjects Assessed	56	15	35	12	21	3	0	0

Task 5a. Obtain informed consent from potentially eligible GW veterans

Recruitment has been ongoing this year and 174 total subjects have contacted GWIC coordinators through print advertising, free newsletters to VSOs or social media outlets. From these contacts, 108 were found eligible to participate in the studies. Those not screened were largely not GW veterans. Of this group, 83 have been scheduled and 71 (56 case, 15 controls) have completed the study protocols at Boston or Miami sites. There is a fair number of participants at the Boston site who were eligible but do not have appointments made. This is due to a number of factors, but most are because these participants are located across the country and heard about our study through social media or word of mouth. At the time of screening, travel was not feasible for these participants. Those participants who screen in have largely completed the studies. We expect to increase recruitment to catch up to the expected recruitment goals of 300 total participants in year 4. We anticipate obtaining these recruitment goals by bringing on the Texas site in the coming month where the goal of 75 participants will be recruited. Table 4 lists the current demographic breakdown of the cohort.

Task 5b. Assess subjects by obtaining demographics, medical history, self-report questionnaires, neuropsychological testing, brain imaging and blood draw and saliva samples.

71 study participants have been assessed to date and subject demographics, exposures and self-reported symptoms are reported below for the study completers. As planned, cognitive assessment data will be analyzed with neuroimaging data to assess for brain-behavior relationships in GWI and will be presented below for the initial sample of study participants. These first participants allowed us to solidify and enhance all recruitment, shipping and sample sharing procedures for the clinical studies. Initial demographic outcomes suggest a fairly diverse cohort of veterans in terms of race and gender as well as self-reported neurotoxicant exposures during the war. Some neurotoxicant exposures are significantly different between the GWI cases and controls including exposure to chemical/biological weapons and reporting wearing pesticide treated uniforms.

Table 4. Subject Demographics N=71			
Demographics	Overall	Cases	Controls
Age at time of study	51	50	53.5
Years of education	15	15	16
Female	14%	18%	0%
Ethnicity			
Caucasian	73%	71%	80%
African American	14%	16%	7%
Other	13%	13%	13%
GW-related Exposures			
Reported PB pill usage	69%	71%	60%
Wore pesticide treated uniforms	62%	70%	33%*
Saw pesticides sprayed/fogged	44%	48%	37%
Chemical/bio weapon exposed	44%	50%	20%*
MFI-20 Fatigue Score (sd)	67 (18)	72 (15)	47 (13)*
McGill Pain Score (sd)	31 (17)	34 (16)	13 (11)*

Table 5 shows the percentage of participants reporting mild traumatic brain injury (TBI) before, during or after the Gulf War. These results suggest that over a third of our cohort to date report sustaining a mild TBI during the Gulf War with nearly half of the cases and none of the controls in this group. Although it would not have been intuitive that GW veterans may show increased rates of TBI due to the short duration of the conflict, these questions were added to the study protocol after rates of mTBI were found to be much higher than expected in the large longitudinally followed Ft. Devens Cohort resurvey study conducted by Drs. Kregel, Janulewicz-Lloyd and Sullivan (Yee et al., 2015).

Table 5. TBI reporting				
mTBI self-report	GWIC Cohort	GWIC cases	GWIC controls	Ft. Devens Cohort
% Pre-War	43	42	47	44
% During GW	37	47	0	31
% Post-War	27	33	7	44

In the Ft. Devens study, Drs. Kregel, Sullivan and Janulewicz-Lloyd reported that GW veterans reporting a history of TBI had a significantly greater risk of reporting many chronic health symptoms (in multiple domains outside of the CNS) and with meeting criteria for chronic multisymptom illness criteria (Fukuda et al., 1998). Thus it was hypothesized that mTBI in addition to GWI neurotoxicant exposures could have resulted in a chronic neuroinflammatory state in these veterans. The GWIC cohort recruited to date includes 30% (n= 21) of veterans who reported ever having a TBI and exposure to chemical/biological weapons during the war. Of this group, GWI cases made up 95% of the dual exposed group (20 cases vs 1 control). Future concurrent studies with the GWIC and the Ft. Devens cohort will further assess this possible multiple-hit hypothesis of GWI in subgroups of veterans and whether the chronic sequelae is worse in these groups. This pattern also fits with recent work reported by other investigators who coined the term post-concussive syndrome (PCS) or post-inflammatory brain syndromes (PIBS) that result after TBI, neurotoxicant exposures (chemotherapy, nerve agents), post-operatively etc. (Rathbone et al., 2015). PIBS/PCS result in chronic

neuroinflammatory signaling of cytokines (IL-1b, IL-6, TNF-a) and have been associated with many behavioral effects including cognitive problems, headaches, fatigue, mood alterations and sleep problems (Rathbone et al., 2015). Initial results are presented below of behavioral correlation of cytokines with cognitive, health symptom and neuroimaging outcomes after a listing of cognitive and survey measures in the main study protocol.

Table 6. Neuropsychological Test Battery for GWI Consortium Study

Test Name	Description	Outcome Measure
I. Executive System Functioning		
Controlled Oral Word Association Test (COWAT)	Spontaneous generation of words from letters F, A and S and animals category.	Total correct words generated
Color-Word Interference Test (DKEFS)	Timed response requiring naming of ink color and inhibiting discordant color-names; measures fronto-executive, selective response and inhibition.	Total Errors
II. Tests of Attention, Vigilance and Tracking		
Trail-making Test (Reitan & Wolfson, 1985)	Timed connect-a-dot task to assess attention and motor control requiring sequencing (A) and alternating sequences (B).	Time to Completion
Continuous Performance Test (Connors CPT3)	Target letter embedded in series of distractors; to assess sustained attention and reaction time.	Reaction Time, Total Omission and Commission Errors
III. Tests of Motor Function		
Grooved Pegboard Test (Klove, 1963)	Speed of inserting pegs into slots using each hand separately; assesses motor coordination and speed.	Raw Score time to completion
Finger Tap Test (manual tapper)	Continuous tapping of computer key with alternate hands; assesses simple motor speed.	Number of taps
IV. Tests of Visuospatial Function		
Block Design Test (WAIS-IV)	Copy picture designs with blocks	Raw Score
Rey-Osterrieth Complex Figure Test	Copy of a complex figure	Total correct out of 36
V. Tests of Memory		
California Verbal Learning Test (CVLT-II; Delis et al., 2000)	List of 16 nouns from 4 categories presented over multiple learning trials with recall after interference; assesses memory and learning strategies.	Total Trials 1-5 Long Delay
Rey-Osterrieth Complex Figure Test	Immediate and delayed recall of a complex figure	Total recall out of 36
VI. Test of Motivation		
Test of Memory Malingering (TOMM)	Test of Memory Malingering (TOMM) is a 50-item visual recognition test designed to help distinguish malingering from genuine memory impairments	Total correct
VII. Mood measure		
Profile of Mood States	65 single-word descriptors of affective symptoms summed on six mood scales.	T – Scores

Table 7. Gulf War Illness Consortium - Survey and Questionnaire Instrument Descriptions

Name	Description
Demographics	Subjects report information on age, education, gender, ethnicity, marital status, GW duty service (active vs. reserve/National Guard), military rank and current military status.
SF36V	Veterans' version of the SF36 which assesses functional health-related quality of life in 8 domains and provides overall summary scores for physical and mental health status.
Kansas Gulf War and Health Questionnaire	Queries veterans about demographics, military and deployment history, and chronic symptoms and diagnoses required to ascertain Kansas GWI and CMI case status.
Medical Conditions	A checklist with 21 medical conditions that the subject is asked to rate if they have ever had the condition, how it was diagnosed (self or doctor) and when it was diagnosed.
Kansas Gulf War Experiences and Exposures	A questionnaire that assesses veteran-reported experiences and exposures during their deployment to the 1991 Gulf War.
Structured Neurotoxicant Assessment Checklist (SNAC)	The SNAC assesses the degree of past and current exposure to neurotoxicants during civilian and military occupations and includes questions pertaining to recent occupational and environmental exposures.
Pittsburg Sleep Quality Index (PSQI)	PSQI assesses sleep quality during the past month. It covers domains of sleep quality, latency, duration, efficiency, disturbances, medications and daytime dysfunction. Total global scores range from 0-21.
Multidimensional Fatigue Inventory Questionnaire (MFI-20)	20 item self-report fatigue instrument that covers general fatigue, physical fatigue, mental fatigue, reduced motivation and reduced activity.
McGill Pain Questionnaire	A pain questionnaire that includes 3 sections including what the pain feels like, change over time and strength of pain. Scores range from 0-78.

Task 5c. Upload neuroimaging data to BUSPH for post-processing of MR images and for data analysis.

MRI scans were obtained from the first 38 study participants at the Boston site and have been post-processed. When the Baylor site begins recruitment, MRI scans will be transferred electronically in either extended DICOM or par/rec format to the Center for Biomedical Imaging at Boston University School of Medicine. Each scan undergoes quality checking that consists of a visual inspection for the presence of noise or artifact as well as a review of scan parameters to ensure that the appropriate ones were used in the acquisition. Scans that fail the quality check are rejected by the study and remediation discussed with the appropriate site investigator. Scans that pass the quality check enter the post-processing pipeline. The first 38 scans have been through the post-processing pipeline and initial correlation results are presented in the sections below.

MRI Imaging: The scanning session include: 1) Three plane TFSE scout scan, 2) a Sense reference Scan, 3) an accelerated high resolution MPRAGE scan acquired in the sagittal plane, 4) a multi-component T2 imaging sequence acquired in the axial plane, 5) a Diffusion Tensor Scan with 32 directions acquired in the axial plane, 6) a resting state functional magnetic resonance imaging scan, and 7) a pCASL sequence obtained while the participant is at rest and 8) a High Angular Resolution Diffusion Imaging (HARDI DTI) scan.

Task 5d. Score neuropsychological tests and upload summary data to DCC for entry, cleaning and analyses.

Data from the first 71 participants has been scored and cleaned. As study protocols are obtained and data is collected, quality control procedures will remain in place

including double entry of data collection forms in the REDCap data collection website, built in range checks and quality control audits of all data collection by the Data Coordinating Center staff and the local BU Administrative Core neuropsychologists. Dr. Toomey also conducts weekly conference calls to review scoring and quality control, as well as regular reviews of data entered and spot checks of any questionable data to ensure data administration and scoring integrity throughout the recruitment period. This will ensure the highest quality data available for analysis.

Task 5e. Send blood and saliva samples to Nova University for analysis of cytokine and chemokine panels and cortisol measurements.

Blood and saliva samples have been sent to NOVA Southeastern University for each of the recently completed 71 study participants. The analysis of cytokine and chemokines has been completed for the first 71 samples and means are reported in Table 8 and in correlation analyses in the section below. Cortisol measurements that will include testing for neuroendocrine and immune alterations and for hypothalamic pituitary adrenal axis abnormalities will occur in larger batch samples. Specifically, blood samples will be sent to NOVA Southeastern University for analysis of proinflammatory cytokine and chemokines, monocyte markers (MCP-1), and nanostring analysis of mRNA and miRNA of proteins related to TLR4 functioning and glial activation including miR-155, miR-21 and miR-146. Multiplex Quansys ELISA system will be used with an existing cytokine platform created by Dr. Klimas' research laboratory. Dr. Klimas' laboratory currently measures 16 cytokines, chemokines and immune markers in plasma. Gene expression and pathways will also be assessed using an Agilent microarray system and quantitative real-time PCR for validation of differentially expressed genes. Preliminary results of cytokine, chemokine, monocyte and lymphocytes between cases and controls are listed below and indicate initial significant differences in several cytokines as well as lymphocytes between the groups. Preliminary correlation analyses of select cytokines with behavioral and neuroimaging outcomes are also shown in figures below.

Table 8 Mean (sd) cytokine (pg/ml) in cases and controls

cytokine	Overall Mean (SD) N=71	Cases Mean (SD) n=56	Controls Mean (SD) N=15	p-value
IL1a	15.69 (58.47)	8.80 (4.45)	41.42 (126.94)	0.05
IL-1 β	26.25 (8.39)	26.45 (8.32)	25.53 (8.89)	0.70
IL-2	16.47 (44.36)	11.38 (1.78)	35.49 (96.61)	0.06
IL-4	5.40 (3.17)	5.58 (3.50)	4.73 (1.21)	0.36
IL-5	2.78 (2.02)	2.87 (1.92)	2.41 (2.38)	0.43
IL-6	2.21 (1.24)	2.19 (1.07)	2.27 (1.78)	0.82
IL-8	23.11 (115.47)	28.26 (129.76)	3.86 (4.29)	0.47
IL-10	9.44 (6.27)	9.33 (6.20)	9.84 (6.73)	0.77
IL-12p70	4.16 (2.78)	3.95 (2.78)	4.92 (2.73)	0.23

IL-13	3.37 (0.56)	3.33 (0.39)	3.54 (0.95)	0.20
IL-15	71 10.91 (1.27)	56 10.99 (1.32)	15 10.62 (1.04)	0.31
IL-17	71 7.01 (13.18)	56 5.38 (0.85)	15 13.08 (28.57)	0.04
IL-23	71 481.53 (1222.79)	56 316.08 (87.83)	15 1099.19	0.02
IFN-Gamma	71 5.45 (27.13)	56 5.94 (30.42)	15 3.63 (6.19)	0.77
TNFA	11.95 (7.49)	12.11 (7.77)	11.37 (6.58)	0.73
TNFB	19.12 (93.82)	22.55 (105.51)	6.32 (7.22)	0.55
TNF RI	426.04 (182.89)	445.30 (189.48)	353.80 (139.55)	0.12
TNF RII	1349.23 (375.75)	1399.23 (393.65)	1161.74 (224.42)	0.05
CRP	0.86 (1.14)	0.91 (1.21)	0.71 (0.95)	0.66
Total Lymphocyte	1.87 (0.57)	1.96 (0.56)	1.43 (0.39)	0.0039
Total Monocytes	0.44 (0.15)	0.46 (0.15)	0.38 (0.10)	0.09

Task 5f. Send additional saliva samples to University of Adelaide for genetic polymorphism analysis

The first batch of 17 saliva samples have been sent to the University of Adelaide for genetic polymorphism studies. Multiple genetic variants of: a) important immune mediators and immune receptors involved in glial activation; and b) pain sensitivity and opioid response to pain; and how these genetic polymorphisms alter pathologies of GWI will be assessed. Variability in immune genetics targets of interest such as the gene encoding for *IL-1B* will be determined in the GWI populations and via comparison with a healthy control population will be related to both the development and severity of GWI symptoms when larger sample sizes are available. The next shipment of ~70 samples will be undertaken by December 2016.

Genomic DNA was isolated from the first 17 saliva samples and genotyped for targets of interest with custom-designed multiplex analysis in Dr. Coller's lab. This provides information regarding genetic variability for 21 single nucleotide polymorphisms (SNPs) in the following genes: *IL-1B*, *IL-2*, *IL-6*, *IL-10*, *TNF-a*, *TGF-b*, *ICE*, *IL-6R*, *TLR2*, *TLR4*, *MD2*, *MYD88*, *BDNF*, *CRP*, and *OPRM1*. In addition, genetic variability in *COMT* (Val108/158Met, rs4680) was studied as this enzyme helps regulate cortical dopamine in the prefrontal cortex and other cortical areas (Tunbridge et al, 2006). Further, the *COMT* (rs4680) polymorphism has been studied in relation to brain volumes, cognitive functioning, pain perceptions and mood dysfunction.

No variant alleles were detected for *MD2* (rs11466004, HapMap Caucasian frequency 2.7%) or *TGF-b* (rs11166314, NCBI European frequency 0%). There was a significant difference in the variant allele

frequency for the *MYD88* rs6853 SNP between GWI and HapMap Caucasian populations, 28 versus 11%, Odds ratio (95% CI) of 3.01 (1.32 – 6.86), $p=0.012$, which may indicate an association between genetic variants of *MYD88* with incidence of GWI. No other significant differences in variant allele frequencies compared to HapMap Caucasian / NCBI European population frequencies were observed (range of p -values: 0.459 - 1.000).

Of the cytokine genetic polymorphisms investigated, there was a significant impact of the *IL-1B* rs1143627 SNP on IL-1 plasma levels (Kruskal-Wallis statistic 6.65, $p=0.0275$), with carriers of the *CT* genotype having the highest IL-1 expression. In addition, the *IL-6R* rs8192284 SNP significantly impacted on IL-6 plasma levels, with carriers of *CC* variant genotype having the lowest IL-6 expression (Kruskal-Wallis statistic 8.78, $p=0.005$). However, as both of these observations are contradictory to previous reports investigating the impact on cytokine expression (for review see Smith & Humphries Cytokine & Growth Factor Reviews 2009, vol 20: 43-59), and also due to the small numbers, these need to be confirmed with further investigation in the larger population prior to making any conclusions.

When considering the impact of genetic variability on functional outcomes, of the SNPs investigated results indicated that *COMT* was significantly associated with two measures of visuospatial functioning including the WAIS-V Block Design test (Figure 1, Kruskal-Wallis statistic 7.6; $p=0.011$) and the Rey Osterreith Complex Figure test Copy condition (Figure 2, Kruskal-Wallis statistic 5.8; $p=0.046$). These findings of two tests of visuospatial functioning being significantly correlated with the *COMT* genetic polymorphism could suggest a vulnerability of some veterans to visuospatial functioning that will be further assessed when larger sample sizes are available and will be added to the cognitive outcome risk factor analyses going forward. There was no relationship between *COMT*, *OPRM1*, *TLR2*, *TLR4* or *MYD88* genotypes and McGill Pain scores.

Figure 1: WAIS-V Block design test

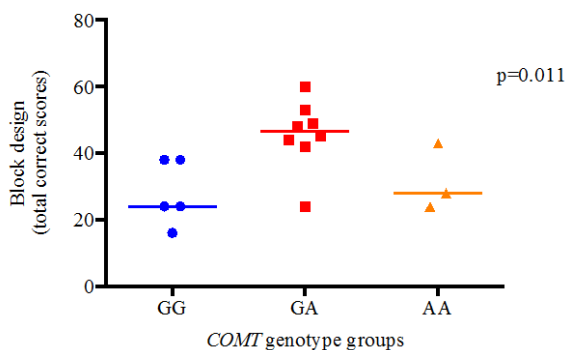
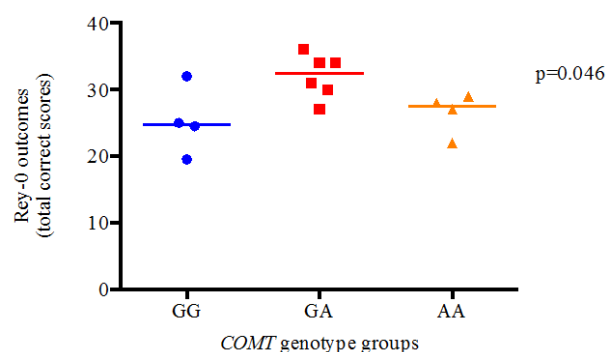


Figure 2: Rey Osterreith Complex Figure test Copy condition



Task 5g. Conduct preliminary analyses of clinical data

The BUSPH Data Coordinating Center has cleaned all current data and prepared the datasets for statistical analysis from the REDCap data capture web database in direct collaboration with the study biostatistician Dr. Heeren, Dr. Sullivan and the study PIs.

The overall aims of this integrated multidisciplinary consortium scientific focus are to (1) To identify validated markers of GW illness by using state of the art neuroimaging, behavioral, genetic and blood markers of neuroinflammatory activation in both clinical and preclinical models that will elucidate targeted and validated treatment strategies (2) To create a Neuroinflammation Risk Profile for GWI (3) To identify viable mechanistic treatments based on identified pathophysiological pathways of GWI that have been validated in preclinical treatment models.

Results of initial Pearson correlation coefficient analyses of cognitive testing outcomes with neuroimaging data presented in Table 9 suggest some interesting potential relationships in structure-function relationships of brain-behavior outcomes. An example includes the correlation of total gray matter volumes and uncorrected errors on the Trail Making Test as well as with hippocampal volumes and slower performance on the DKEFS color-word interference tasks. Other examples of the visual memory task, the Rey-Osterrieth Complex Figure immediate recall task appearing correlated with hippocampal volume would be expected and was shown in our prior studies (Sullivan et al., 2013). The verbal memory task, the CVLT-II list learning task being correlated with subcortical gray matter volume on short delay recall is also an interesting finding. However, the lack of significance with the Rey-Osterrieth and CVLT-II delayed recall performances will be further assessed when larger samples sizes are obtained and data is adjusted for common covariates including age, education and gender. Table 10 presents Pearson Correlation coefficients of brain volumes with cytokine data. Results to date in GWI cases indicate some interesting patterns with white matter volumes and cytokines (IL13, IL15, TNF-RII) that will be further assessed when larger samples are available for comparison.

Table 9. Pearson Correlations of Select Cognitive and Neuroimaging Outcomes Coefficients Prob > r under H0: Rho=0				
Cognitive test (correlation coefficient, p value)	Hippocampus	CerebralWhiteMatterVol	SubCortGrayVol	TotalGrayVol
Trail Making Test: Trail B Uncorrected Errors	0.33590 0.08	0.25820 0.18	0.31029 0.10	0.41819 0.02*
D-KEFS Color-word interference: Trial 3 Total time	-0.36163 0.05*	-0.00435 0.98	-0.16456 0.40	-0.29153 0.13
D-KEFS Color-word interference: Trial 4 Total time	-0.36425 0.05*	0.07004 0.72	-0.20855 0.28	-0.23277 0.23

CVLT-II: # Correct in SD recall	0.00930 0.96	-0.13055 0.50	-0.37998 0.04*	-0.17601 0.37
CVLT-II: # Correct in LD recall	-0.01521 0.93	-0.15481 0.43	-0.21461 0.27	-0.09884 0.61
REY: Total Copy	-0.11244 0.56	0.20721 0.29	-0.04688 0.81	0.08343 0.67
REY: Total Immediate	0.38017 0.04*	0.11936 0.54	-0.02795 0.88	0.14219 0.47
REY: Total Delay	0.17109 0.38	0.05907 0.76	-0.23559 0.27	-0.06723 0.73

Table 10. Pearson Correlations of neuroimaging and cytokine outcome coefficients

	IL1a	IL1b	IL_6	IL_13	IL_15	IL_17	IL_23	TNF_RII	TotMono
MRI: Hippocampus	0.12408 0.52	-0.00794 0.96	-0.31126 0.10	-0.21447 0.27	-0.15643 0.42	-0.00781 0.96	0.15907 0.41	-0.30592 0.13	0.26706 0.18
MRI: Cerebral White Matter Volume	-0.25212 0.1956	-0.08374 0.67	-0.31842 0.09	-0.37925 0.04*	-0.53707 0.003*	0.35013 0.06	0.23147 0.23	-0.49822 0.01*	0.53920 0.004*
MRI: Sub Cortical Gray Volume	-0.06863 0.72	-0.03522 0.85	-0.37516 0.04*	-0.23939 0.21	-0.22822 0.24	-0.12504 0.52	0.20370 0.29	-0.32407 0.11	0.15178 0.45
MRI: Total Gray Volume	0.13808 0.48	-0.11730 0.55	-0.31143 0.10	-0.20542 0.29	-0.19109 0.33	-0.04947 0.80	0.32905 0.08	-0.28241 0.17	0.29547 0.14

Table 11 presents Pearson correlation coefficient analyses of cytokine cognitive outcomes that suggest potential important immune-behavior outcomes particularly with respect to verbal memory performance on the CVLT-II list learning task delayed recall correlating with IL-15 cytokine levels. In addition, significantly slower reaction times on the Connors CPT3 task are significantly correlating with IL-13 and IL-6 levels such that as the cytokines increase, the reaction time on this computerized task also increases, suggestion worse performance on this task. These relationships will be further compared when larger sample sizes are available to assess by case-control status. Although IL-15 has recently been reported to be associated with fatigue ratings, we did not find this correlation in our sample to date but we did find a correlation with VAS worse report of pain and IL1 levels (Younger et al., 2015). All results will be compared when larger study samples are available and less speculative conclusions can be drawn.

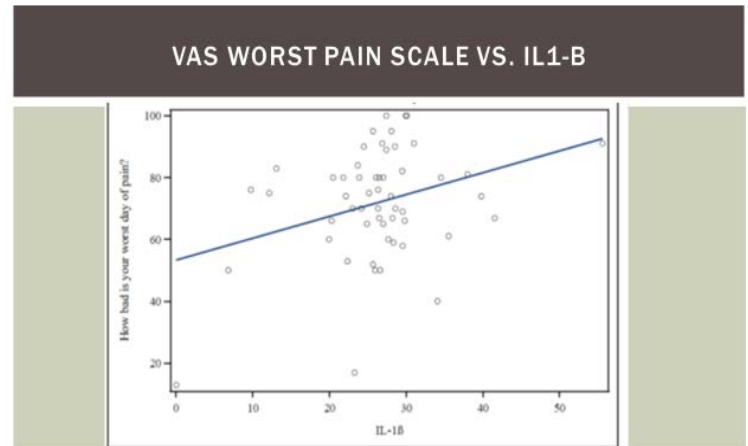


Table 11. Pearson Correlation of Select Cognitive and Health Symptom Outcomes and Cytokines.

Cognitive Tasks	IL_5	IL_6	IL_8	IL_10	IL_12p70	IL_13	IL_15	CRP	TotLymph	TotMono
Trail Making Test: Trail B Time	0.10281 0.45	0.01612 0.90	-0.18144 0.18	0.31361 0.01*	0.05027 0.71	-0.02904 0.83	-0.11471 0.39	-0.03866 0.83	0.02632 0.85	0.38426 0.005*
Trail Making Test: Trail B Uncorrected Errors	-0.18982 0.16	-0.12463 0.36	-0.02948 0.82	-0.19113 0.15	-0.26589 0.04*	-0.00253 0.98	-0.13498 0.32	0.03506 0.85	-0.07159 0.61	0.05593 0.69
CPT 3: Hit Reaction Time T Score	0.07394 0.59	0.23502 0.08	0.24027 0.07	-0.05293 0.70	-0.20114 0.14	0.26089 0.05*	0.14379 0.29	-0.02462 0.89	0.26716 0.05	0.00208 0.98
CPT 3: Hit Reaction Time Raw Score	-0.01420 0.91	0.33753 0.01*	0.24362 0.07	-0.04717 0.73	-0.24401 0.07	0.24689 0.06	0.11763 0.39	-0.00151 0.99	0.27111 0.05*	0.05460 0.70
D-KEFs Stroop: Trial 4 Total time	0.29336 0.03*	-0.12996 0.35	-0.05960 0.67	0.20515 0.14	0.12606 0.37	-0.11342 0.42	-0.27386 0.04*	-0.42367 0.02*	0.25990 0.07	0.04826 0.74
FTT: Dominant Hand mean	0.21190 0.12	-0.25717 0.06	-0.07831 0.57	0.03310 0.81	0.25878 0.05*	-0.00625 0.96	-0.05955 0.66	0.20333 0.28	-0.18975 0.18	0.10540 0.46
FTT: Non Dominant Hand mean	0.14856 0.28	-0.22718 0.09	-0.07760 0.57	0.04180 0.76	0.20584 0.13	-0.00348 0.98	0.05353 0.70	0.14163 0.45	-0.10967 0.44	0.15777 0.27
CVLT-II: # Corrcr in SD recall	-0.14581 0.28	0.01047 0.93	0.23441 0.08	-0.02045 0.88	-0.08303 0.54	0.16723 0.22	0.27023 0.04*	-0.06254 0.74	0.14487 0.31	0.07041 0.62
CVLT-II: # Correct in LD recall	-0.23707 0.08	-0.05825 0.67	0.20714 0.12	0.06776 0.62	0.02886 0.83	0.08626 0.53	0.34249 0.01*	-0.06265 0.74	0.19522 0.16	0.07727 0.58
REY O: Total Immediate recall	0.15020 0.27	0.06243 0.65	0.07038 0.61	-0.07565 0.58	-0.03970 0.77	-0.06291 0.65	0.18702 0.17	0.35848 0.05*	0.07936 0.58	-0.00719 0.96
REY O: Total Delay recall	0.21542 0.12	0.04697 0.73	0.12429 0.37	-0.06156 0.66	-0.07936 0.57	0.08851 0.52	0.27148 0.04*	0.24813 0.19	-0.02504 0.86	0.03746 0.79

Task 6. RECRUITMENT AND ASSESSMENT OF BOSTON CSF AND PET STUDIES (months 24-42)

The Boston call-back studies are currently being planned and almost ready to be started. Specific plans and estimated start dates are listed below.

Task 6a. Perform lumbar punctures to obtain cerebrospinal fluid markers of neuroinflammation in 50 GW Veterans – Preparatory planning has included obtaining lumbar puncture kits and needles necessary for the study and an INR machine has been purchased to check for proper coagulation in potential LP study participants. A new neurologist, Dr. Anna Cervantes was identified to perform the lumbar punctures. The first two lumbar punctures have been completed and recruitment efforts for this follow up study will continue in the coming year.

Task 6b. Perform positron emission tomography (PET) scanning with novel EAAT2 ligand in partnership with RIO pharmaceuticals in 15 GW veterans. - Plans are underway to begin IRB and FDA IND approval submissions for the RIO PET imaging pilot study. Drs. Sullivan, Killiany, Gerdes and Cox have had several planning calls and hope to submit IRB and FDA approvals in late fall and then to HRPO when local approvals are in place. It is hoped that first participants will be run in the early spring.

Task 6c. Perform FDG-PET scan imaging with 30 GW veterans after a computerized CPT cognitive challenge task. – Because many marker differences identified in GWI have been shown after challenge tasks, a FDG-PET imaging pilot study will also be conducted after a continuous performance test (CPT) of information processing and sustained attention task 30 GW veterans will be assessed for differences in glucose utilization when compared with GW veteran healthy controls. Initial meetings have been conducted with Drs. Sullivan, Killiany and the new director of BU radiology to plan for the initial FDG PET studies. Recruitment has begun for this follow up study. Necessary equipment has been obtained a final protocol has been agreed upon and clinical approvals are now obtained for final approvals through radiology. The first participants will be scheduled in the next month.

Task. 7. Interim Analyses, Grant Submission, and Annual Reporting (Months 18-42)

7a. Data entry of all questionnaires, evaluations and quality control measures will be ongoing

Data entry of all questionnaires and evaluations has been ongoing in as close to real time as possible. The Data Coordinating Center also tracks missing and inconsistent data. The latest data integrity report is listed below.

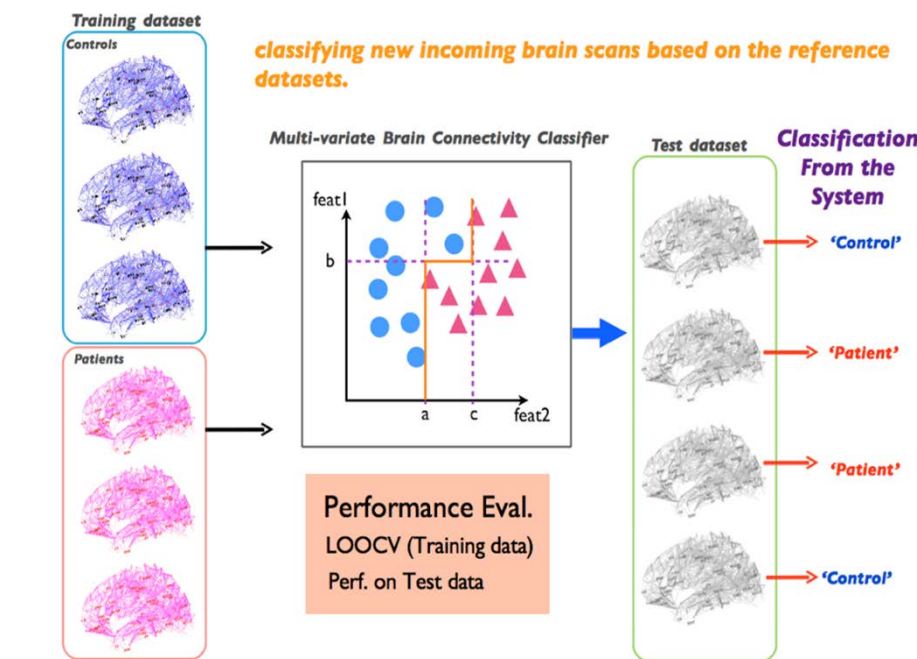
GWIC: Gulf War Illness Consortium Data Integrity Report Data as of October 7, 2016

<i>Site</i>	<i>Out of Range Data</i>	<i>Missing Data Fields</i>	<i>Invalid Logic</i>
Boston	0.00% (1 of 27222)	0.09% (74 of 85656)	0.07% (1 of 1463)
Miami	0.00% (0 of 8412)	0.04% (11 of 26404)	0.00% (0 of 451)
Total	0.00% (1 of 35634)	0.08% (85 of 112060)	0.05% (1 of 1914)

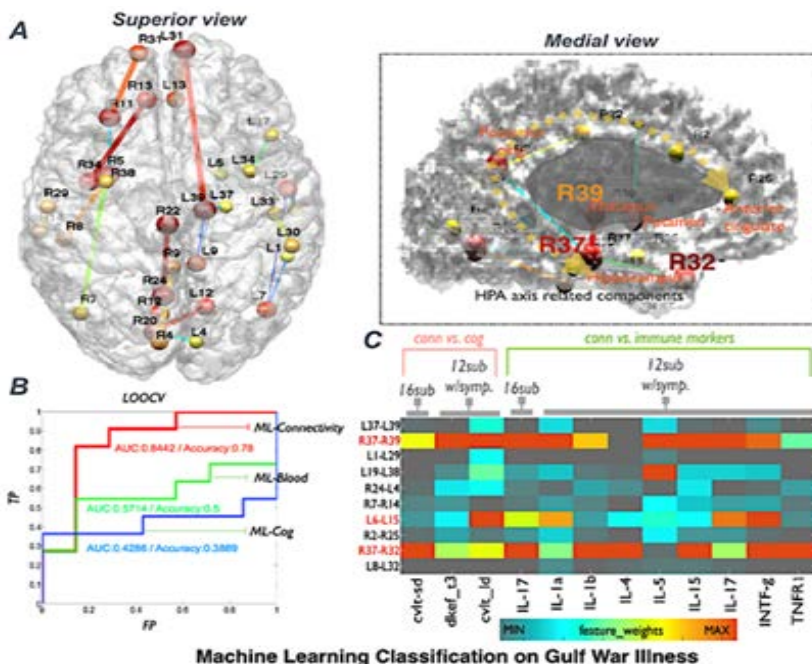
7b. Interim Statistical analyses of data obtained from cognitive evaluations, blood markers, neuroimaging and questionnaire data will be performed periodically.

Interim analyses of machine learning multi-modal approach to brain connectomics is described below.

Brain Connectomics and pilot machine learning analysis: To generate the connectivity patterns we used high angular resolution diffusion magnetic resonance imaging (HARDI) data from 18 veterans with GWI and 12 elderly control subjects. The GWI subjects also had cognitive and blood-immune markers. Regional brain connectivity was defined based on the existence and amount of white matter tracts between 68 cortical and subcortical regions. For the white matter connectivity assessments, BCF which is based on a machine learning



algorithm was applied to define a subset of brain connections specific to GWI. Brain connections selected from the group assessments were then compared to cognitive and immune assessments in both univariate and multivariate general linear modeling. Since 8 (1 GW control and 7 GWI) datasets were newly acquired during the building process, we used these data as a test dataset for additional classification performance evaluation. In this work, similar performance test results on both validation tests were expected only if the classifier did not over-estimate the patterns or biased to the training dataset. Brain connections selected from the group assessments were then compared to cognitive and immune assessments in both univariate and multivariate general linear modeling schemes.



Brain Connectomics machine learning analysis:

Panel A. Veterans with GWI had significant connectational alterations in the regions including 'the thalamus (L39) - frontal pole (L31)', 'the posterior cingulate (R22) - precuneus (R24)', 'the hippocampus (L37) - thalamus (L39)' and 'the insula (R34) - medial orbitofrontal (R13)' connections. In terms of the brain gray matter, both classifications pinpointed the regions related to the hypothalamic-pituitary-adrenal (HPA) axis, such as the hippocampus and medial portion of the anterior prefrontal cortex.

Panel B. Classification between GWI and GW controls showed an accuracy of 78% (area under the curve-AUC 0.8442) in the LOOCV. In the test data validation, it showed 75% on classifying GWI. Test data validations revealed 40% in cognitive markers and 20% in blood markers.

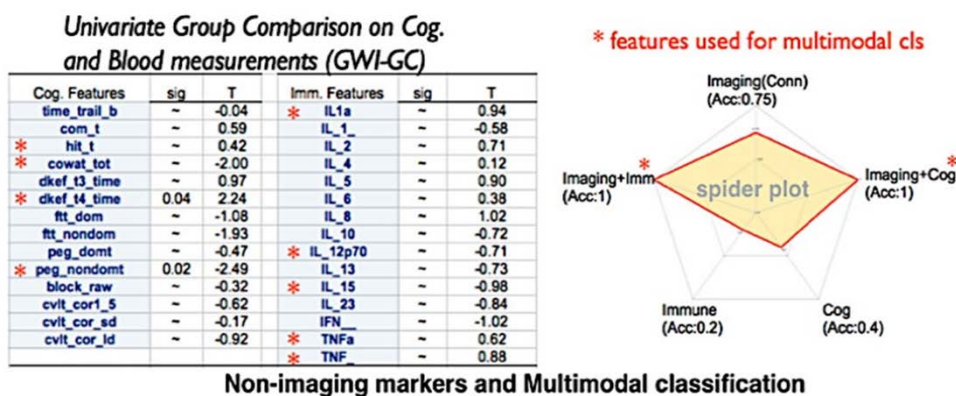
Panel C. Some of these markers had clear relationships to the brain connectomic features.

Machine learning techniques were used on GWI diffusion MRI data and the classification performance was very encouraging and was comparable to that of MCI classification based on either diffusion MRI. The spatial distribution of the brain connectomic features differed from that of AD or MCI suggesting that different diseases appear to display different signature machine-level patterns.

Enhanced classification performance from multimodal biomarkers

Combining some of either blood immune or cognitive markers with the neuroimaging markers (red asterisks in the left table) helped to improve the overall classification results on the test dataset (spider plot).

Combining neuroimaging markers with either blood immune or cognitive measures revealed 100 % accuracy in classifying GWI in both classifiers.



Some of the immunogenomic markers provide meaningful supplementary information to the brain connectomic markers in classifying GWI. Deterioration in one component of the brain can be involved in multi-symptom illness and therefore, it is expected that improved classification of GWI can be obtained by adding independent information such as immune markers (cytokines, chemokines), which can reflect different types of neurotoxicant exposures or sub-symptoms of GWI.

7c. Grant submissions to relevant funding agencies for further collaborative studies based on initial results and preliminary data targeted toward treatment strategies will be ongoing.

Since the GWIC was funded, 11 grants have been submitted from our investigators and 10 have been funded to date. See the next sections regarding specific grants and collaborative aims. Many of these studies will utilize the GWIC biorepository stored samples for further analyses of biomarkers and gene-exposure outcomes.

7d. Annual reports of progress will be written. Annual reports have been written for the past 3 years with corresponding quarterly reports in between.

KEY RESEARCH ACCOMPLISHMENTS

- Obtained final IRB approvals for Baylor Medical College, Nova Southeastern University, Miami VA and Boston University. Obtained HRPO approvals for Nova Southeastern University and Boston University and Baylor Medical College. Obtained Exempt status at University of Adelaide.
- Trained all clinical staff in-person on neuropsychological testing and structured clinical interviews and obtained all necessary equipment for three clinical sites.
- Data Coordinating Center has programmed online surveys in REDCap web data capture software, developed a bar-coded specimen tracking system and a consortium website for subject recruitment.

- Subject recruitment for clinical studies has begun and 71 study participants have been assessed.
- Sub-studies have begun recruitment and are ongoing.
- Initial clinical studies have begun and are showing promising results for:
 - Brain-behavior relationships in study cohort as shown by structure – function correlations
 - Brain-immune relationships as shown by cognitive outcomes – cytokines correlations
 - Immune-behavior relationships as shown by symptom reporting – cytokine correlations
 - Building a Neuroinflammatory Risk Profile of GWI based on early study results from machine learning analyses.
- Obtained final and continuing protocol approvals by the respective IACUCs and ACURO for the preclinical animal research sites at CDC/NIOSH, NIH, Temple University and University of Colorado.
- Initial preclinical studies have begun and are showing promise results for:
 - Behavioral effects of the neuroinflammatory model of GWI in rats
 - For axonal transport alterations in the in-vitro GWI model that show CORT exacerbates DFP and promising results for tubacin treatment.
 - For altered myelination in the GWI rat model and cholinergic signaling related to myelination in the in-vitro model.
 - Chronic neuroinflammatory phenotype shown in later time points in rodent models and promising initial results for minocycline treatment.

CONCLUSIONS

This multi-institutional collaboration of highly qualified GWI researchers from public universities, federal agencies, and the private sector, provide an unprecedented opportunity to more fully elucidate the underlying pathobiology of Gulf War illness in one integrated model that once proven, will lead to focused treatment trials that can be quickly implemented.

The central hypothesis for the pathobiological mechanisms of GWI in this consortium includes chronic neuroinflammation as a result of initial glial activation and then priming of glial responses that cause stronger and longer responses that do not shut off the chemical cascade of proinflammatory cytokines and chemokines that cross-talk between the immune system and the brain. This could result in a lasting multisystem illness affecting many body systems, as seen in GWI.

Improved understanding of the role of glial activation in chronic pain states has given rise to rapidly expanding efforts to identify pharmaceuticals that specifically focus on glial functions. The growing availability of treatments of this type gives particular urgency to our efforts to determine the extent to which glial activation and central cytokine activation explain the symptoms of GWI. In order to specifically address the research gaps outlined by the IOM and the RAC reports with regard to biomarker identification and pathobiology of GWI, this research team is characterizing disease symptoms and validating and improving pathobiological markers based on collective prior clinical and preclinical studies and leveraging longitudinal cohorts and stored blood samples with the ultimate goal of identifying targeted and effective treatments for GWI. Initial preliminary results suggest that the consortium animal model of GWI is correlated with behavioral alterations seen in clinical studies including altered visuospatial memory functioning and that

chronic neuroinflammation is present in the DFP + CORT model. Myelin studies show an increase in myelin basic protein at early time points and now at later time points that will be assessed for and mechanism. As these preclinical models are further developed, they will be correlated with clinical studies to identify markers of the illness and targets for therapeutic intervention. Early preliminary clinical study results suggest brain-immune-behavioral outcome correlations that bode well for the derivation of a neuroinflammatory risk profile of GWI, diagnostic marker development and targeted therapeutic strategies in the near future.

PUBLICATIONS, ABSTRACTS and PRESENTATIONS

BU investigators presented 2 posters and an oral presentation as part of symposium on Gulf War Illness at the International Association of Chronic Fatigue Syndrome (IACFS) in Ft. Lauderdale, FL, October 27-29, 2016 and at the INIM pre-conference at NOVA University.

CDC will be presenting 4 posters related to our GWI work at the 46th Annual Society for Neuroscience meeting in San Diego, CA, November 12-16, 2016.

CDC will be presenting data on GWI as part of a panel discussion on sickness behavior and disease at the 50th Annual Winter Conference on Brain Research in Big Sky, MT, January 28-February 2, 2017.

CDC will be presenting data on GWI as part of a symposium on sickness behavior and disease at the 56th Annual Society of Toxicology meeting in Baltimore, MD, March 12-16, 2017.

BUSPH and Duke University investigators will be presenting a poster related to the collaborative CNS autoantibody study at the 56th Annual Society of Toxicology meeting in Baltimore, MD, March 12-16, 2017.

Publications

Dutta, D.J., Woo, D.W., Lee, P.R., Pajevic S., Bukalo, O., Wake, H., Huffman, W.C., Basser, P.J., Lazarevic, R.D. (2016) Myelin plasticity regulated by vesicular release of protease nexin1 from perinodal astrocytes. *Science* (in revision).

Fields, R.D., Dutta, D.J., Belgrad J., Robnett, M. (2016) Cholinergic Signaling in Myelination. *Glia* (accepted).

L. Abdullah, JE Evans, U Joshi, G Crynen, J Reed, B. Mouzon, S. Baumann, H Montague, Z. Zakirova, T. Emmerich, C. Bachmeier, **N. Klimas, K. Sullivan,** M.Mullan, G. Ait-Ghezala and F. Crawford. Translational analysis of long-term decreases in mitochondrial lipids in a mouse model of Gulf War Illness. *Toxicology*. Accepted.

O'Callaghan, JP, Michalovicz, LT, Kelly, KA. Supporting a Neuroimmune Basis of Gulf War Illness. *EbioMedicine*. 2016 Oct 26. pii: S2352-3964(16)30498-4. doi: 10.1016/j.ebiom.2016.10.037. [Epub ahead of print]

O'Callaghan, J.P., Kelly, K.A., Locker, A.R., Miller, D.B., & Lasley, S.M. (2015). Corticosterone Primes the Neuroinflammatory Response to DFP in Mice: Potential Animal Model of Gulf War Illness. *Journal of Neurochemistry*, 133, 708-721

Yee, M., Seichepine, D., Januelwicz Lloyd P., **Sullivan, K.,** Proctor, SP & **Krengel, M.** Traumatic brain injury, health and rate of chronic multisymptom illness in veterans from the 1990-1991 Gulf War. *Journal of Head Trauma Rehabilitation*. Aug 19, 2015 (epub ahead of print).

Yee, M., Seichepine, D., Janulewicz Lloyd P., **Sullivan, K. & Krengel, M.** History of Pre-War Brain Injuries Influences Total Current Health Symptoms in a Cohort of 1990-1991 Gulf War Veterans. Abstract. 43rd Annual Meeting Abstracts, Journal of the International Neuropsychological Society, Supplement 1, March 2015: 8.

Seichepine, D., Yee M., Janulewicz Lloyd P., **Sullivan, K. & Krengel, M.** Frequency of Traumatic Brain Injuries in a Cohort of 1990-1991 Gulf War Veterans. Abstract. 43rd Annual Meeting Abstracts, Journal of the International Neuropsychological Society, Supplement 1, March 2015: 14.

Maule, A., **Janulewicz, P., Krengel, M.,** White, RF, Judd, S., Cirillo, J., **Sullivan, K.** A meta-analysis of Self-reported Neurological and Neuropsychological Symptoms in Gulf War Veterans. Journal of the International Neuropsychological Society, Supplement 1, March 2015: 9.

Seichepine, D., Yee, M., **Janulewicz-Lloyd P., Sullivan, K & Krengel, M.** Chronicity of Health Symptoms in the Ft. Devens Cohort. International Neuropsychological Society, 42nd Annual Meeting Abstracts, Journal of the International Neuropsychological Society, Supplement 1, February 2014; 165.

Sullivan, K., Krengel, M., Janulewicz, T., and Chamberlain, J. An overview of toxicant exposures in Veteran cohorts from Vietnam to Iraq. In Amara, J. ,Hendricks, A.(eds) (2013) *Military medical care: From predeployment to post- separation*, Abingdon: Routledge.

Abstracts

DH Woo, DJ **Dutta**, W Huffman, M Robnett, PR Lee, K **Sullivan**, R **Killiany**, J **O’Callaghan**, RD **Fields**: Role of glia in the pathophysiology of Gulf War Illness. Abstract & Poster. Society for Neuroscience, San Diego, CA Oct 2016.

BB Koo, JP O’Callaghan, K Kelly, L Michalovicz, K Sullivan, and RJ. Killiany. High-order diffusion MRI on the potential animal model of Gulf war illness. Abstract and Poster. International Association of Chronic Fatigue Syndrome, Ft. Lauderdale, FL Oct 2016.

BB Koo, K Sullivan, J Cirillo, M Krengel, R Toomey, P Janulewicz Lloyd, Z Barnes, L Steele, N Klimas, and RJ Killiany. Brain Connectivity Patterns in Gulf-War Illness: Relationship with Cognitive and Immune Measurements. Abstract and Poster. International Association of Chronic Fatigue Syndrome, Ft. Lauderdale, FL Oct 2016.

L. Abdullah, T. Emmerich, JE Evans, U Joshi, J Reed, G. Laco, V. Mathura, **K. Sullivan, N. Klimas, M. Mullan, F. Crawford.** Application of lipidomics for identifying novel blood biomarkers of Gulf War Illness. Abstract and poster. International Association of Chronic Fatigue Syndrome, Ft. Lauderdale, FL Oct 2016.

M.B. Abou-Donia, **K. Sullivan**, L. Conboy, E. Kokkotou: Serum Autoantibodies to Neural-Specific Proteins as Objective Biomarkers for Gulf War Illness. Abstract & Poster. Society of Toxicology, New Orleans, LA March 2016.

Kelly KA, Locker AR, Michalovicz LT, Miller DB, O’Callaghan JP: Exploration of the gulf war illness phenotype in a mouse model challenged with LPS at long term time points. Abstract & Poster. Society of Toxicology, New Orleans, LA March 2016.

Revitsky AR, Kelly KA, Miller DB, Lasley SM, O'Callaghan JP: Organophosphate-induced Neuroinflammation, With and Without Corticosterone Pretreatment, Is Not Due to Acetylcholinesterase Inhibition. Abstract & Poster. Society of Toxicology, New Orleans, LA March 2016

Michalovicz LT, **Locker AR, Kelly KA, Miller DB, O'Callaghan JP:** Corticosterone priming of the neuroinflammatory response to acetylcholinesterase inhibitors results in overexpression of TLR2 and downstream targets, but not activation of the NLRP3 inflammasome. Abstract & Poster. Society of Toxicology, New Orleans, LA March 2016

Michalovicz LT, **Locker AR, Kelly KA, Miller DB, O'Callaghan JP:** Chronic corticosterone primes the brain response to select neuroinflammatory agents by overexpression of toll-like receptor 2 and S100A8: A potential role of microglia. Abstract & Poster. Society for Neuroscience, Chicago, IL October 2015

Locker AR, Kelly KA, Michalovicz LT, Miller DB, O'Callaghan JP: Corticosterone primes the neuroinflammatory responses to Gulf War Illness associated exposures: Effects of irreversible vs reversible acetylcholinesterase inhibitors. Abstract & Poster. Society for Neuroscience, Chicago, IL October 2015

Kelly KA, Locker AR, Michalovicz LT, Miller DB, O'Callaghan JP: Phenotype comparisons of ALDH1L1 BAC-TRAP mice under control and neurotoxic (MPTP) conditions. Abstract & Poster. Society for Neuroscience, Chicago, IL October 2015

Revitsky AR, Kelly KA, Miller DB, Lasley SM, O'Callaghan JP: Pyridostigmine bromide suppresses neuroinflammation induced by DFP. Abstract & Poster. Society of Toxicology, San Diego, CA March 2015

Kelly KA, Revitsky AR, Miller DB, Lasley SM, O'Callaghan JP: Chronic glucocorticoid and nerve agent DFP exposures produce a neuroinflammatory model of Gulf War Illness without neurodegeneration. Abstract & Poster. Society of Toxicology, San Diego, CA March 2015

Presentations

K. Sullivan. Brain Immune Interactions in Gulf War Illness: Cytokines and Cognition in US Military Veterans. Abstract and presentation. International Association of Chronic Fatigue Syndrome, Ft. Lauderdale, FL Oct 2016.

K. Sullivan. Understanding Gulf War Illness: Brain-Immune Biomarkers, Cognitive Functioning and Treatment Development Strategies 25 Years after the War. Symposium. International Neuropsychological Society (INS) in London, July 6th- 8th

K. Sullivan. Brain Immune Interactions in Gulf War Illness: Cytokines and Cognition in US Military Veterans. Symposium. International Neuropsychological Society (INS) in London, July 6th- 8th

K. Sullivan Cytokines, Cognition and Gulf War Illness in a US Military Veteran Cohort. Symposium. International Neuropsychological Society (INS) in London, July 6th- 8th

Abou-Donia M. Screening for novel objective central nervous system biomarkers in veterans with Gulf War Illness. Symposium. International Neuropsychological Society (INS) in London, July 6th- 8th

Krengel, M. Exploring the association between cognitive symptoms and exposures in a cohort of 1990-1991 US Gulf War Veterans. Symposium. International Neuropsychological Society (INS) in London, July 6th- 8th

Sullivan, K. Neurotoxicity of Gulf War Deployment: The Neuropsychological and Neuroimaging Correlates. Invited lecture. Miami VA Gulf War 25th anniversary event. March 29, 2016.

Sullivan, K. Neurotoxicity of Gulf War Deployment: The Neuropsychological and Neuroimaging Correlates. Boston University School of Public Health, Introduction to Toxicology (EH768, guest lecture), Boston, MA, March, 16, 2016.

Sullivan, K., Klimas, N. Committee and Panel Discussion: ‘how to discussion’ for GWI Biomarker Research, Research Advisory Committee on Gulf War Veterans’ Illnesses; Spring Meeting, Washington, DC, September, 2014.

O’Callaghan, J., Sullivan, K. Committee and Panel Discussion: ‘how to discussion’ for GWI animal research, Research Advisory Committee on Gulf War Veterans’ Illnesses; Spring Meeting, Washington, DC, April, 2014.

Seichepine, D., Yee M., **Janulewicz Lloyd P., Sullivan, K. & Kregel, M.** Chronicity of Health Symptoms in the Ft. Devens Cohort. International Neuropsychological Society, 42nd Annual Meeting, Seattle, WA, February 2014.

Steele, L. Committee and Panel Discussion: ‘how to discussion’ for GWI Case Criteria Research Advisory Committee on Gulf War Veterans’ Illnesses; Winter Meeting, Washington, DC, January, 2014.

Seichepine, D., Yee, M., **Janulewicz Lloyd P., Sullivan, K., Proctor, S., & Kregel, M.** Traumatic Brain Injury and Health Status of Veterans from the 1990-1991 Gulf War. Boston University Second Annual Joining Forces TBI/PTSD Event, Boston, MA, December, 11, 2013.

Sullivan, K. RAC-GWVI Treatment Development Discussion. Research Advisory Committee on Gulf War Veterans’ Illnesses; Summer Meeting, Washington, DC, June, 2013.

Sullivan, K. Neurotoxicity of Gulf War Deployment: The Neuropsychological and Neuroimaging Correlates. Boston University School of Public Health, Introduction to Toxicology (EH768, guest lecture), Boston, MA, March, 26, 2013.

INVENTIONS, PATENTS AND LICENSES – N/A

REPORTABLE OUTCOMES

Newly Funded Studies

- PET PBR28 study funded with MGH investigators (Loggia: PI; GW130100)
- Human induced pluripotent stem cells (IPSC) stem cell grant funded with Drexel, BU and other GWIC investigators (Baas PI; Sullivan site PI; GW140086)
- D-cycloserine pilot treatment study funded with Boston University investigators (Toomey PI; Sullivan co-I) (GW140069)
- CNS autoantibody grant submitted with Duke investigators (Sullivan Initiating PI; Abou Donia Partnering PI; GW140140)
- Tai Chi treatment trial with Boston VA investigators (Niles PI; Sullivan site PI; VA CSR&D)
- CoQ10 Phase III trial 4 site study submitted to VA with Miami VA, GWIC and other investigators
- Ft Devens cohort cognitive, blood and neuroimaging assessment of brain antioxidant glutathione levels (Kregel, Initiating PI; Sullivan Partnering PI; GW140140)
- PON1 study with GWIC investigators and San Francisco VA investigators (GW150037)
- Gulf War Women’s Health Cohort with Augusta University investigators (GW150116)
- Lipidomics and proteomics study with Roskamp Institute investigators (GW150056)
- +naltrexone pain treatment New Investigator proposal with U-Colorado investigators (GW150187)

Newly Funded Studies

- **Title: An in-vivo investigation of Brain Inflammation in Gulf War Illness with Integrated PET/MR imaging (PI: Loggia)**
Supporting agency: Department of Defense (GW130100)
Performance period: 9/1/14-8/31/17
Level of funding: \$1,026,352
Brief description of project's goals/ Specific aims: The overarching objective of this study is to demonstrate the pathological occurrence of microglial activation in the brains of patients with Gulf War Illness and document the effects of this activation on Gulf War Illness symptomatology and brain physiology using new imaging approaches. The project's three specific aims are (1) to demonstrate in vivo activation of microglia in veterans with Gulf War Illness, (2) to demonstrate the association between microglial activation and alterations in brain physiology and anatomy, and (3) to demonstrate an association between microglial and neural activity with symptom severity; i.e., fatigue, pain, disability, depression, and anxiety.
- **Title: Novel Autoantibody Serum and Cerebrospinal Fluid Biomarkers in Veterans With Gulf War Illness (PI: Sullivan and Abou-Donia)**
Supporting agency: Department of Defense (CDMRP/GWIRP) (GW140140)
Performance period: 9/1/15-8/31/18
Level of funding: \$449,751
Brief description of project's goals/ Specific aims: We hypothesize that following neural damage in GWI there is loss of cells, breakdown of the blood brain barrier leading to leakage of specific neuronal and glial proteins into circulation, with subsequent formation of their autoantibodies that can still be quantified. Specific Aims include: 1) To determine whether IgG-class autoantibodies for CNS markers are present in the blood sera of veterans with GWI compared with healthy GW veteran controls or compared with patients with irritable bowel syndrome (IBS). 2) To determine whether AChE inhibitor exposures during the war (i.e. low-dose sarin, pesticides, PB) are associated with IgG-class autoantibodies for CNS markers in veterans with GWI compared with veterans without GWI. 3) To determine whether IgG-class autoantibodies for CNS markers in veterans with GWI correlate with neuroimaging and cerebrospinal fluid markers in veterans with GWI compared with veterans without GWI. 4) To determine whether prior CNS insults (mTBI) are associated with Ig-G class autoantibodies for CNS markers in GW veterans with GWI compared with GW veteran controls.
- **Title: D-cycloserine –A Novel Treatment for Gulf War Illness (PI: Toomey)**
Supporting agency: Department of Defense (GW140069)
Performance period: 7/1/15-6/30/17
Brief description of project's goals/ Specific aims: The overall objective of this GWI treatment study is to compare the efficacy of the randomized double-blind clinical treatment trial of DCS for the treatment of GWI. We hypothesize that the treatment trial of DCS will be effective in improving cognitive functioning in GW veterans with GWI, particularly memory functioning. Specific Aims: 1) To compare efficacy of the novel therapeutic approach of DCS in improving cognitive functioning in GW veterans with GWI. 2) To examine different time points in order to determine optimal timing of doses of DCS for positive effects on cognitive functioning. 3) To compare efficacy of DCS in improving mood, health symptoms and quality of life measures in GW veterans with GWI.
- **Title: Novel Interventions for Gulf War Veterans' Illnesses (PI: Niles and Mori)**
Supporting Agency: DVA CSR&D
Performance Period: 10/1/2015 – 9/30/2020
Level of funding: \$1,600,000

Brief Description: This randomized trial will establish the effectiveness of a Tai Chi mind-body treatment in Veterans with GWI. Tai Chi is a traditional Chinese mind-body therapy that has been practiced for centuries. In the last decade, the PIs have demonstrated that Tai Chi can improve both physical health and psychological wellbeing in patients with a variety of chronic conditions. The long-term goal is to develop a safe, readily available, mind-body treatment to reduce pain and other chronic symptoms and enhance wellness in Veterans with GWI.

➤ **Title: Microtubule abnormalities underlying Gulf War Illness in neurons from human induced pluripotent cells (PI: Baas)**

Supporting agency: Department of Defense (GW140086)

Performance period: 7/1/15-6/30/17

Level of funding: \$1,026,352

Brief description of project's goals/ Specific aims: The objective is to develop new immortal lines of pluripotent cells derived from peripheral blood mononuclear cells (PBMCs) of GW veterans themselves, so that an altered microtubule hypothesis (as well as other GWI hypotheses) can be tested. The other objective is to assess whether available microtubule-active drugs can correct these abnormalities and provide treatments for GWI. Specific aims are: 1. Develop human neurons or glial cells derived from human induced pluripotent stem cells (hiPSCs), originating from 15 GW veterans with GWI and 15 healthy GW veteran controls. 2. Develop a microtubule-based strategy to treat impaired nervous system functions in GWI.

Newly Submitted Studies

- Machine learning New Investigator proposal with Boston University (BU) investigators
- BChE + PON1 biomarker epidemiological New Investigator proposal with BU investigators
- BChE and white matter basic science New Investigator proposal with NIH investigators
- Epigenetic DNA methylation study submitted with Naval Research Lab investigators
- Rituximab treatment trial proposal with Nova Southeastern University investigators

The consortium website (<http://sites.bu.edu/gwic>) is continually updated to disseminate news about new papers and studies related to Gulf War Illness.

OTHER ACHIEVEMENTS – N/A

REFERENCES

1. Banks, C.N., & Lein, P.J. (2012). A review of experimental evidence linking neurotoxic organophosphorus compounds and inflammation. *Neurotoxicology*.
- 2 G. Broderick, A. Kreitz, J. Fuite, M.A. Fletcher, S.D. Vernon, N. Klimas, A pilot study of immune network remodeling under challenge in Gulf War Illness, *Brain, behavior, and immunity* 25 (2011) 302-13.
3. Chao, L.L., Abadjian, L., Hlavin, J., Meyerhoff, D.J., Weiner, M.W. (2011). Effects of low-level sarin and cyclosarin exposure and Gulf War Illness on brain structure and function: a study at 4T. *Neurotoxicology*, 32, 814-22.
4. Heaton KJ, Palumbo CL, Proctor SP, et al. (2007). Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. *Neurotoxicology*. 28(4):761-769. <http://www.ncbi.nlm.nih.gov/pubmed/17485118>
5. Hutchinson MR , et al. (2008) Proinflammatory cytokines oppose opioid-induced acute and chronic analgesia. *Brain, Behavior, and Immunity*. 22(8): 1178-1189.
6. Institute of Medicine. (2010). *Gulf War and Health: Volume 8. Health Effects of Serving in the Gulf War*. Washington, DC: National Academies Press. <http://www.iom.edu/Reports/2010/Gulf-War-and-Health-Volume-8-Health-Effects-of-Serving-in-the-Gulf-War.aspx>
7. Milligan, E.D., & Watkins, L.R. (2009). Pathological and protective roles of glia chronic pain. *Nature reviews Neuroscience*, 10, 23-36.
8. Milligan ED., Chacus M., Gazda LS., Armstrong C., Wang H., Tracey KJ., Maier SF., Watkins LR.,(2001) Sciatic inflammatory neuritis (SIN): behavioral allodynia is paralleled by peri-sciatic proinflammatory cytokine and superoxide production. *Journal of Peripheral Nervous System* 6(3):111-29.
9. O'Callaghan, JP., Kelly, KA., Locker AR., Miller DB., Lasley SM. (2015) Corticosterone primes the neuroinflammatory response to DFP in mice: potential animal model of Gulf War Illness. *Journal of Neurochemistry* 133(5):708-21. doi: 10.1111/jnc.13088
10. Rathbone, AT., Tharmaradinam, S., Jiang S, Rathbone MP, Kumbhare DA. (2015) A review of the neuro- and systemic inflammatory responses in post concussion symptoms: Introduction of the “post-inflammatory brain syndrome” PIBS. *Brain, Behavior and Immunity*. 46; 1-16.
11. Research Advisory Committee on Gulf War Veterans' Illnesses. (2008). *Gulf War illness and the Health of Gulf War Veterans: Scientific Findings and Recommendations*. Washington, DC: US Government Printing Office. http://www1.va.gov/RAC-GWVI/Committee_Documents.asp
12. Rivest, S. (2009). Regulation of innate immune responses in the brain. *Nature Reviews. Immunology*, 9(6), 429-439. doi:[10.1038/nri2565](https://doi.org/10.1038/nri2565)

13. Sullivan K, Krengel M, Proctor SP, Devine S, Heeren T, White RF. (2003). Cognitive functioning in treatment-seeking Gulf War veterans: pyridostigmine bromide use and PTSD. *Journal of Psychopathology and Behavioral Assessment*. 25(2):95-103.
14. Spradling, K.D., Lumley, L.A., Robison, C.L., Meyerhoff, J.L., Dillman, J.F. (2011). Transcriptional responses of the nerve agent-sensitive brain regions amygdale, hippocampus, piriform cortex, septum, and thalamus following exposure to the organophosphonate anticholinesterase sarin. *Journal of neuroinflammation*, 8, 84.
15. Toomey R, Alpern R, Vasterling JJ, Baker DG, Reda DJ, et al. (2009). Neuropsychological functioning of U.S. Gulf War veterans 10 years after the war. *J Int Neuropsychol Soc*. 15(5):717-29. doi:10.1017/S1355617709990294
16. Turnbridge, EM., Weickert CS., Kleinman JE., Herman MM., Chen J., Kolachana BS., Harrison PJ., Weinberger DR. (2006) Catechol-o-methyltransferase enzyme activity and protein expression in human prefrontal cortex across the postnatal lifespan. *Cerebral Cortex* 17(5):1206-12.
17. Watkins, L. R., Hutchinson, M. R., Ledeboer, A., Wieseler-Frank, J., Milligan, E. D., & Maier, S. F. (2007). Norman Cousins Lecture. Glia as the "bad guys": implications for improving clinical pain control and the clinical utility of opioids. *Brain, Behavior, and Immunity*, 21(2), 131-146. doi:[10.1016/j.bbi.2006.10.011](https://doi.org/10.1016/j.bbi.2006.10.011)
18. Watkins, L. R., Hutchinson, M. R., Rice, K. C., & Maier, S. F. (2009). The "toll" of opioid-induced glial activation: improving the clinical efficacy of opioids by targeting glia. *Trends in Pharmacological Sciences*, 30(11), 581-591. doi:[10.1016/j.tips.2009.08.002](https://doi.org/10.1016/j.tips.2009.08.002)
19. Whistler, T., Fletcher, MA., Lonergan, W., Zeng, XR., Lin, JM., LaPierre, A., Vernon, S. & Klimas, N. (2009). Impaired Immune Function in Gulf War Illness. *BMC Medical Genomics*, 2-12. <http://www.biomedcentral.com/1755-8794/2/12>
20. Yee, M., Seichepine, D., Januelwicz Lloyd P., Sullivan, K., Proctor, SP & Krengel, M. Traumatic brain injury, health and rate of chronic multisymptom illness in veterans from the 1990-1991 Gulf War. *Journal of Head Trauma Rehabilitation*. Aug 19, 2015 (epub ahead of print).
21. Zhang, D., Xiaoming, H., Qang, L., O'Callaghan, JP, Hong, JS. (2010). Astroglisis in CNS Pathologies: Is there a role for microglia? *Mol. Neurobiology*, doi:[10.1007/s12035-010-8098-4](https://doi.org/10.1007/s12035-010-8098-4).
22. Zhang, F., Liu, J., & Shi, J. (2010). Anti-inflammatory activities of resveratrol in the brain: role of resveratrol in microglial activation. *European Journal of Pharmacology*, 636(1-3), 1-7. doi:[10.1016/j.ejphar.2010.03.043](https://doi.org/10.1016/j.ejphar.2010.03.043)
23. Zhang, Q., Zhou, X.D., Denny, T., Ottenweller, J.E., Lange, G., LaManca, J.J., Lavietes, M.H., Poller, C., Gause, W.C., Natelson, B.H. (1999). Changes in immune parameters seen in Gulf War veterans but not in civilian with chronic fatigue syndrome. *Clinical and diagnostic laboratory immunology*, 6, 6-13.

Appendix A. Brain Immune Interactions as the Basis of Gulf War Illness: Gulf War Illness Consortium (GWIC)

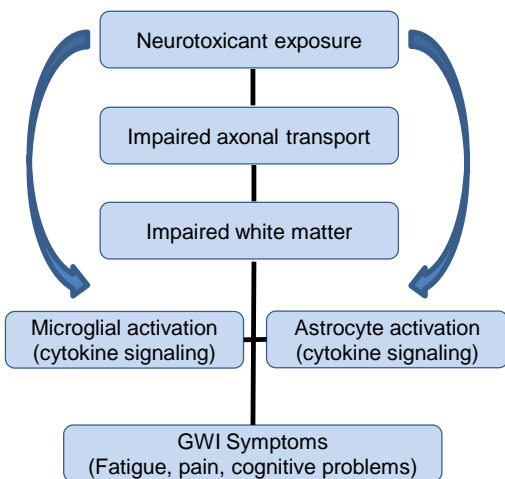


Award Number: GW120037 / W81XWH-13-2-0072

PI: Dr. Kimberly Sullivan

Org: Boston University Medical Campus

Award Amount: \$4,888,851

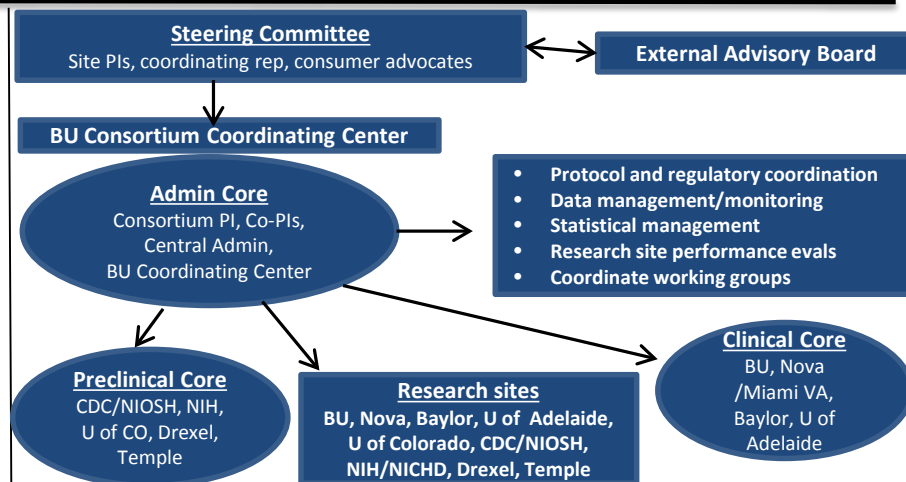


Hypothesized GWI Mechanisms

Approach

A series of clinical and preclinical studies to test whether GWI is related to chronic brain-immune activation and chronic inflammation.

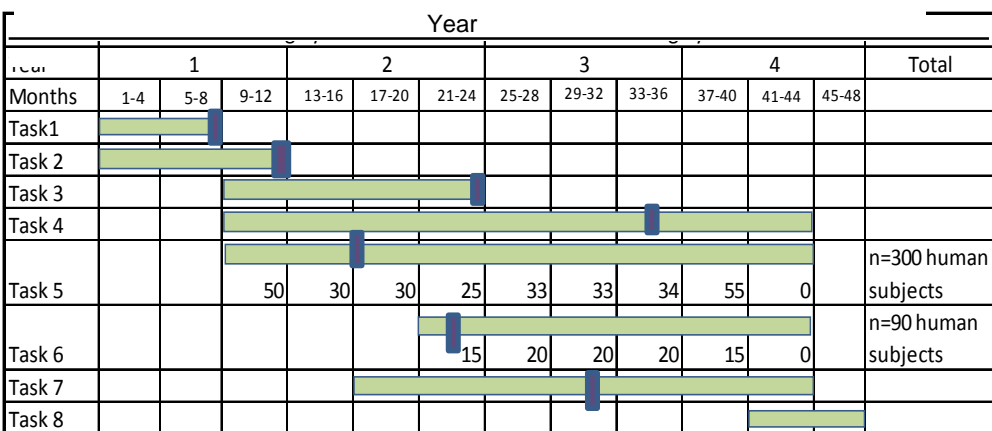
- Clinical case-control studies will be conducted in parallel at 3 sites — Boston, Miami, and Central Texas and will include a total of 300 Gulf War veterans.
- Markers in blood, cerebrospinal fluid, brain imaging (advanced MRI, PET scans) and memory testing will be examined.
- Parallel preclinical studies will evaluate persistent effects of GW neurotoxins in *in vitro* and rodent models of GWI.



Accomplishments: 1-IACUC and ACURO approvals are in place for all sites 2-IRB and HRPO approval obtained from BU, Baylor, Miami VA and NOVA. 3-Data and tracking systems, websites finalized 4-Laboratory methods established for immunologic assays. 5-Preclinical studies ongoing at all sites, have initial results and pilot treatments started. 6- Subject recruitment ongoing and first 71 subjects completed. 7- Six grant applications were funded for further collaborative research efforts. 8 -Conference symposium, abstracts and manuscripts submitted.

Sept 2013 Start

Timeline



Goals/Milestones

FY13 Goal – Obtain necessary authorization prior to human/animal studies and preparation for consortium clinical/preclinical studies

- ✓ Kick-off meeting with GWIRP staff and study PIs
- ✓ Protocol preparation and initiation of approvals for animal/human use (Task 1)
- ✓ Creation of databases/manuals and data use agreements (Task 2)
- ✓ Prepare rodent dosing models and *in vitro* cell models (Task 3)

FY14 Goal –

- ✓ Perform preclinical cell/animal studies (Task 4)
- ✓ Screening, recruitment, assessment of GW veterans at 3 sites (Task 5)

FY15 Goal – Recruitment and assessment for Boston CSF/PET studies (Task 6-7)

FY16 Goal – Statistical and validation analysis (Task 7-8)

Next External Advisory Board meeting scheduled for November 2016